

24 January 1980

Curing Europe's biological death-wish

LAST WEEK an apparently European firm, Biogen, incorporated in Luxembourg and with a laboratory in Geneva, announced that it has successfully engineered *Escherichia coli* to produce interferon — currently touted both as an anti-viral and as an anti-cancer agent, after some small trials have shown remissions; larger trials are on the way. So does Europe rejoice at its technological breakthrough?

Unfortunately not, for Biogen stockholdings are held almost exclusively in North America. The major exceptions are the seven European scientists involved; with two Americans, the scientists hold one third of the stock. The largest individual stockholder is International Nickel, a Canadian-based mineral company, with the drug firm Schering-Plough — which has bought the international licence to exploit the Biogen creation — in second place. A handful of other bodies have holdings of under 10%. Hence the remark of Charles Weissman, who co-engineered the interferon-producing bug, quoted in this issue (page 323), that it was a shame that European industry had not taken greater interest in Biogen. Apparently those who were forming the company approached a number of firms in Europe, particularly in Switzerland and West Germany — but none was interested in a share in the business.

The fault, then, was doubly Europe's: first, it was a fault not to attempt to draw on its own potential bioengineers through its own 'Biogen' — no one was adventurous enough to set it up, though many had the idea; and second, it was a fault to ignore a ready-made opportunity — Biogen itself — when it was offered.

Furthermore in the UK a number of scientists have been attempting for three years to interest venture capital and the government in a British contract research organisation, akin to Biogen, to no avail. Some who were active in that attempt are now members of Biogen. The National Research Development Corporation, which might be expected to take a lead in such matters, seems content merely to exploit. Whereas four of six major revenue-earners are biotechnical, its past and present biological development projects are outnumbered by the non-

biological 10:1.

Neither do European politicians have much to praise each other about. When it began to be possible to make high fructose syrup economically from maize starch using immobilised enzymes, the Council of Ministers of the European Community — the highest and most political authority — slapped a punitive levy on the process, killing its development (which was already underway in Britain), in the name of protecting beet growers.

No wonder that a draft version of the forthcoming Royal Society report on biotechnology recommends that Europe puts its house in order. Biotechnology must not invite penal levies, says the draft, merely because Community policy is in the grip of interests which "resist the cheaper and better products of this new technology, particularly where these threaten to perturb existing patterns of agriculture". Common Agricultural Policy legislation which threatens biotechnology must be revised; and arrangements reached whereby agricultural surpluses can be turned to good use as biotechnical feedstocks.

In this and other ways the report is a strong blast of good sense which the British government — and the Community — would do well to respect. Recession, when individual firms are without the funds to invest, is exactly the moment when governments can move in legitimately to pick up the bill for long-term, strategic research. This also happens to be exactly the moment when technology is finding a new lease of life, through microcircuits and now biotechnology. The nations which will dominate the economics of the 21st century will be the ones that have invested in the new ideas — the ones that have the new processes underway and the new talent in their industries. Engineered bugs and microchips will be the coalfields of the next century: and they are not to be found, but created.

Two great opportunities are shortly to be forthcoming in biotechnology: the £16 million research programme proposed for the EEC which the European Commission should not foolhardily reject; and the recommendations of what will, we hope, be a not too diluted version of the draft report we describe this week (page 324). Europe and the UK must embrace both of them. □



United States

Regulators loosen up on carcinogen standards

Labour unions and the chemical industry have locked the US Occupational Safety and Health Administration in a dispute over translating carcinogenicity data into appropriate regulatory controls. **David Dickson** reports

LAST week Dr Eula Bingham, head of the US Department of Labour's Occupational Safety and Health Administration (OSHA), unveiled the final version of the agency's new scheme to identify and regulate carcinogens in the industrial workplace.

The scheme was originally proposed more than two years ago as a way of streamlining the regulation of cancer-causing substances. The intention was to set out broad criteria for deciding how to deal with certain types of data in determining whether a substance is carcinogenic. Mr Ray Marshall, Secretary for Labour, has hailed it as a "landmark policy" and a "tremendous step forward" in protecting workers from cancer.

But in attempting to find a position that fulfils OSHA's congressional mandate while also minimising its vulnerability to the inevitable legal challenges, the agency has loosened some of its original proposals, adding the criticism of labour unions to that of the chemical industry.

The industry, while pleased with some of the new flexibility, remains unhappy with the scheme. And the American Industrial Health Council (AIHC), representing a number of leading chemical companies such as Dow Chemical and Monsanto, has already asked a federal appeal court to review the regulations, claiming that they "lack scientific validity" and impose an unreasonable economic burden.

But OSHA is now facing opposition from one of its previous supporters, the American Federation of Labour — Conference of Industrial Organisations (AFL-CIO), which has filed its own suit in the District of Columbia, claiming that by relaxing some "cornerstone" provisions the agency has weakened the scheme's effectiveness.

In broad outline, OSHA's new policy for regulating potential suspected carcinogens (see box for definition) is comparable with that first suggested in 1977. In particular, the agency has stuck to its proposal to place suspected carcinogens in two principal categories. The first contains substances for which there is good scientific evidence of carcinogenicity; exposure to such substances is to be reduced to lowest feasible levels, or eliminated if substitutes can be found. The second category contains substances for which there is "suggestive" evidence of carcinogenicity.

As previously, OSHA states that, in evaluating claims of carcinogenicity, it will

not accept a 'threshold' hypothesis, and will still give greater weight to positive animal studies showing evidence of carcinogenicity than to 'non-positive' human studies showing no such evidence.

However the agency has shifted from laying down strict, formal criteria for making such assessments, to relying more on the judgment of scientists, in particular through the use of scientific review panels.

Thus several alterations have been made in the criteria used to classify a substance as belonging to category 1. For example, it was originally proposed that a substance could be included in this category if it was shown to be carcinogenic in one animal bioassay, the results of which were subsequently replicated.

OSHA received various objections to this criterion, some claiming that at least two animal tests were necessary, others that a single, well-conducted test should suffice, and that to require replication or confirmation would unreasonably delay regulatory action.

Responding to these criticisms, OSHA

A 'potential suspected carcinogen':

Any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms in humans or in one or more experimental mammalian species, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory or dermal exposure, or any other exposure which results in the induction of a tumour at a site other than the site of administration.

A positive result in a short-term test would be:

Positive results in assays for two or more of the following type of effect:

- The induction of DNA damage and/or repair
- Mutagenesis in bacteria, yeast, *Neurospora* or *Drosophila melanogaster*
- Mutagenesis in mammalian somatic cells
- Mutagenesis in mammalian germinal cells
- Neoplastic transformation of mammalian cells in culture.

has agreed to amend its criteria so that now a substance can meet the definition of a potential term bioassay "where the results are in concordance with some other scientifically evaluated evidence of a potential carcinogenic hazard" or in a single mammalian species "in which the secretary determines the requirement for concordance evidence is not necessary".

Evidence of 'concordance' may include positive results from testing in the same or other species; or positive results in short-term tests (OSHA has reaffirmed its previous position that such tests can be used to confirm, but not to substitute for, bioassay data, claiming much of the criticism of these tests to be "unsubstantiated"); or the induction of tumours at injection or implantation sites (despite protests from industry, OSHA has again refused to distinguish between malignant and non-malignant tumours as evidence).

In contrast to its earlier proposal, OSHA has decided to drop two further classificatory proposals, one covering substances for which there was a suspicion, but no hard evidence, of carcinogenicity, and the other for substances not occurring in US workplaces.

More controversially, the agency has also decided that preliminary classification in category 1 will not, as previously proposed, automatically require the Secretary of Labour to issue an 'emergency temporary standard' requiring companies using the substance in question to reduce exposure to lowest feasible levels, at least until firm regulations have been established.

Rather, OSHA will publish at the beginning of each year a list of potential candidates for each category. Preliminary estimates indicate that there could be about 150 chemicals in category 1 and 350 in category 2. Every six months OSHA will then select about ten substances from each category for priority attention, with the Secretary of Labour having the power to impose emergency standards if he felt that the chemical poses a "grave danger" and that such a standard is needed.

This added flexibility has angered the labour unions, strong supporters of automatic standards for category 1 substances. OSHA points out that it may lack the legal powers to carry out such a policy — and officials add that if OSHA did introduce such a rigid ruling, there might be a reluctance to place substances into this category because of legal challenges.

But the AFL-CIO is disputing this, complaining that by dropping what it calls "indispensable provision", OSHA has removed the "cornerstone" of its cancer policy, in conflict with the requirements of the Occupational Safety and Health Act of 1970 requiring it to take all steps necessary

to protect the health of US workers.

The chemical industry has, in general, welcomed the new flexibility in the rules — although it still considers them to be too harsh. For example industry rejects OSHA's criteria for accepting epidemiological evidence of non-carcinogenicity.

And the industry remains critical of the costs. Dr Bingham, in presenting the new scheme — scheduled to be published this week in the *Federal Register* — said that some studies now showed that the costs of

cancer exceeded \$15 billion a year.

But she repeated the agency's opposition to using cost-benefit analysis in its rule-making rather than restricting itself to calculations of economic feasibility. Allegations that OSHA was trying to create a "risk-free" environment, regardless of cost, "conveniently overlook that Congress charged OSHA with protecting workers to the extent feasible" she said.

But the industry is not convinced. Significantly the AIHC has chosen to file a suit

requesting federal review of the new regulations with the fifth circuit court of appeals in New Orleans. It was this court which, two years ago, rejected OSHA's proposed new benzene exposure standards on the grounds that the agency had used cost-benefit techniques in reaching its conclusions. The case is now before the Supreme Court for final arbitration. A decision against OSHA could have a devastating effect on OSHA's effectiveness. □

US to announce \$700 million ocean-drilling research programme

In a unique experiment in cooperation with private industry, writes **David Dickson**, the US government and leading oil companies are about to announce plans for sharing the costs of a 10-year research programme to investigate the Earth's continent margins

WHEN President Carter presents his 1981 budget proposals to the US Congress next Monday, he is expected to include plans for one of the biggest and most ambitious research projects ever sponsored by the National Science Foundation: a 10-year programme to investigate the geology of the ocean floor.

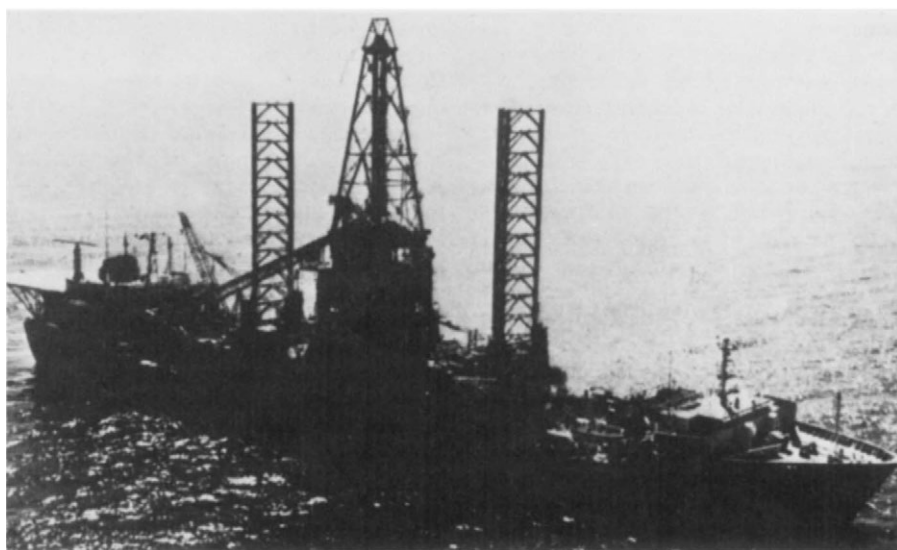
The programme offers potential pay-offs both in terms of increasing understanding of the history and dynamics of the Earth's crust, and of assessing the exploitable resources — in particular ore and hydrocarbon deposits — which the crust contains.

And in a unique arrangement largely credited to the President's science adviser, Dr Frank Press, the estimated \$700 million costs of the programme are to be shared equally by the federal government and private oil companies.

Final details of the agreements are yet to be reached. But it is understood that at least eight companies have agreed in principle to participate in the scheme. Several others have indicated their interest.

At a later stage, it is also intended that the programme will include the participation of research teams from other countries. Britain, France, West Germany, Japan and the Soviet Union each contribute \$1 million to the NSF's current deep-sea mining programmes, and several have expressed interest in participation in the new project, as have Canada, Mexico and Australia.

Speaking at the Woods Hole Oceanographic Institution in Boston, Massachusetts, last week, Dr Press said that the administration intends to make use of the ship *Glomar Explorer* for the ocean



Glomar Explorer: to be made use of in the new programme

drilling project. Originally built by the Central Intelligence Agency to attempt to retrieve a Soviet submarine from the Pacific floor, the vessel has more recently been used by private corporations for investigating the potential of sea-bed minerals. It is considered almost ideal for the type of operation currently envisaged.

The idea of using the *Glomar Explorer* for ocean-floor drilling has been actively discussed in the geological research community for several years, especially since interest has focused on the ocean margins, the zone between the continental shelf and the deep ocean basin.

For the past 12 years, the NSF has supported a major deep-sea drilling project from the smaller *Glomar Challenger*. But although the 'challenger' has produced much important science, leading to increased understanding of plate tectonics and other geological processes, it has been limited in its scope.

In particular, the *Challenger* lacks the equipment to prevent a blow-out should it hit a pocket of pressurised oil or natural gas during experimental drilling. This has restricted its ability to investigate the continental margins, since it is in these regions that such pockets are likely to occur, rather than in the central ocean basin.

To explore such regions, while at the same time minimising the potential danger resulting from a blow-out, drilling will have to be carried out with the aid of a 'riser' — a protective shell surrounding the drill — as well as other appropriate preventive equipment.

The general desirability of a large-scale programme of ocean drilling has been supported by various groups who have studied the idea, in particular by a special committee of the Joint Oceanographic Institutions for Deep Earth Sampling (JOIDES) whose report on the matter was issued in 1977.

Further reports prepared for the NSF endorsed the scientific importance of a deep-sea drilling project, designed to reach the ocean floor at a depth up to 4,000 metres and drill 7,000 metres below that, and indicated that such a programme was technically feasible, with the use of the *Glomar Explorer* as the most promising platform to drill from.

In order to study the overall importance of the programme in the context of national scientific priorities — a move requested by Congress in reviewing the Foundation's 1979 budget request — the NSF established a top-level committee under Dr Guyford Stever which recommended last summer that ocean

margin drilling "should be given high priority consideration for approval" in the NSF's 1981 budget.

The report also supported moves to gain support from private corporations in the US. This strategy has been actively canvassed by the Office of Science and Technology Policy, which Dr Press directs. And although corporate involvement had been objected to by some scientists on the grounds that it could steer the project away from basic research priorities, it soon became clear that the financial climate would not support the project on federal funds alone.

Last autumn both Dr Press and Dr Richard Atkinson, Director of the NSF, spent considerable time and effort talking to the heads of major US oil companies soliciting their support. The reactions were generally favourable, and in response to a formal approach, a number have now agreed in principle to help match the federal funding.

Negotiations have at times been delicate, given in particular the price sector's traditional reluctance to cooperate directly with the federal government, rather than on a contract basis. And various measures have been discussed to make the deal more attractive.

For example, it is expected that companies will not, at this stage, be required to commit themselves for the full ten years, but will have the option of pulling out after one year or after five years. It is also likely that the first location chosen for investigation will be off the North Atlantic coast of the US, an area of interest to the petroleum companies.

Details of how the scientific programme will be organised are also under discussion.

One likely arrangement is that the scientific efforts will be coordinated by the Joint Oceanographic Institutions Inc, a consortium of nine institutions involved in oceanographic research. There would also be three policy review boards, one established by the National Academy of Sciences, one by the Department of Defense, and one by various private institutions and corporations.

Total funding request for the next financial year is expected to be about \$20 million, half coming from the NSF — whose total deep-sea drilling budget would increase from \$18 million to about \$32 million, and half from the oil companies.

So far, despite some reservations about the corporate involvement, the reaction of most geologists to the scientific opportunities has been enthusiastic. Congressional reaction, however, has been more mixed.

Representative Edward Boland, for example, Chairman of the House Appropriations Committee responsible for the NSF budget, has already written to President Carter urging full support for the project. But others are more sceptical, one congressional staff member pointing out that the cost per hole will be at least an order of magnitude greater than with the Challenger.

But the administration is convinced that it is sufficiently important to merit substantial support. "This will be an important scientific venture with possible implications for future resource discovery" said Dr Press. "We believe it is also a good example of government-industry cooperation, the kind of cooperation that we must see more of, in more fields, in the coming years". □

Laser fusion project to be halted?

PRESIDENT Carter is expected to recommend to Congress next week a halt to construction work on the \$200 million Nova laser fusion project, which began last year at the University of California's Lawrence Livermore Laboratory as a successor to the Shiva system first operated in 1978.

Administration officials in Washington confirmed last week a story appearing in the *Washington Post* which claimed that the Office of Management and Budget had recommended that funding for Nova be dropped from the President's budget request for the fiscal year 1981.

The agency is said to have been concerned partly about the cost of the project, and partly about growing uncertainties about whether glass laser technology can meet the efficiency of a commercially viable source of power.

Congress authorised \$23 million for Nova in 1979 — and a further \$56 million for 1980. The foundations for the new machine are currently being laid near Shiva.

A number of recent reports, however, have indicated that the project should be slowed until more is known of the physics of laser/pellet interaction, and of the engineering problems involved.

Officials at Lawrence Livermore are making no public comment until the final budget is published next week. But if funding for Nova is dropped, this is likely to be firmly resisted by the armed services committees in both houses of Congress, which support laser fusion research on account of its potential military applications.

Soviet Union

Science planners and the Press tackle the 'two cultures'

SOVIET science planners have taken up the "two cultures" controversy of CP Snow. A new Press "discussion", launched last week by the prestigious *Literaturnaya Gazeta* will deal with the falling popularity of the applied sciences and its possible effect on the Soviet economy.

The new campaign, which will include expert articles and readers comments, is entitled "Applied science; prestige and people". It is intended to look into the factors hindering the implementation in production practice of new scientific results. This is not a new theme — the slow rate of implementation of R&D into industry has for some years been a recurrent complaint of planners and Party leaders alike.

Recently, however, the problem has been seen in a somewhat wider context. In December 1979, Moscow radio put out a Press-round up on the dirft of Soviet

school-leavers away from science. Instead of the "infatuation" with science and technology, which had characterised university applications for the past two decades, there was now, said the commentator, a significant increase in applications for medicine, economics and teacher-training and journalism courses. So long as this drift from science reflected a genuine response of young people to "spiritual values", he concluded, it was, on the whole, a good thing.

Literaturnaya Gazeta, however, is less approving of the drift. In the course of creation of a scientific society, it observes, a "certain stereotype mode of thinking" has grown up, which considers that "applied research in the interests of this or that branch" is "second-rank and non-prestigious". The two opening articles of this discussion set out to contradict this assumption. The first, by A Savelev, a

sector-chief of the Central Scientific Research Institute of the Sewing Industry, cites C P Snow to the effect that many "pure" scientists fail to grasp the fact that many engineering problems are in no way inferior as regards explicitness and strictness to those with which they (the scientists) are concerned. And Boris Smagin, a leading science writer says that many Soviet scientists including Vernadskii, Bardin and Kurchatov "always placed the interests of science on the same level as the interests of the national economy".

The problem of prestige, however, is, as Smagin admits, a "mysterious" one. More precisely, in the words of an editorial footnote, the "prestige of the profession of applied-scientist is a social-psychological concept." The Press discussion of the role of prestige, explain the editors, is intended to focus attention on this. **Vera Rich**

Switzerland

Engineered *E. coli* produce interferon

THE announcement, last week, by Professor Charles Weissmann of the University of Zurich that his laboratory, on behalf of Biogen, had managed to recover biologically active interferon from bacteria containing human interferon DNA, establishes Biogen as a company that means business. Although the yield of interferon so far obtained from the genetically engineered bacteria is far too low for commercial production, Professor Weissmann thinks that "if things work out as hoped, we should have a high yielding strain within six to twelve months and we could then move relatively fast".

Until recently interferon has only been available in very small quantities with a purity of less than 1%. It has been produced by laboriously purifying human lymphocytes taken from blood donated for transfusion in Finland. A number of very small scale clinical trials have suggested that interferon is effective not only as an antiviral agent but also in the treatment of neoplastic disease. Because of the success of the initial trials there is now a tremendous demand for sufficient interferon to set up bigger trials.

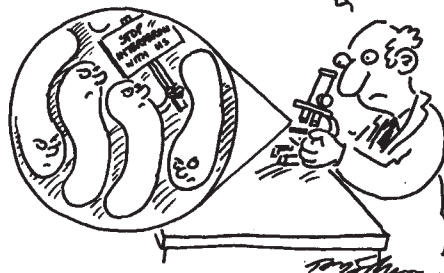
The man behind the Finnish programme is Dr Kari Cantell. He provided Biogen with the human lymphocytes from which its genetic engineering approach started. The first step in that approach was to prepare complementary single stranded DNA from a crude mixture of messenger RNA extracted from virus-stimulated human lymphocytes.

Since there was no way to isolate the sequences corresponding to the interferon gene from the DNA, it was necessary to clone the DNA at random (using the plasmid pBR322 as vector and *Escherichia coli* as the bacterial host) and then to screen the resultant bacteria for any that contained the interferon gene.

Five thousand bacterial clones were screened by extracting their plasmid DNA. The plasmids containing interferon DNA

were determined from their ability to direct the production of biologically active interferon when injected into oocytes of a frog. Having identified individual bacterial clones containing interferon genes, Professor Weissmann's team established that the bacteria were producing interferon with much the same biological and chemical characteristics as authentic human lymphocyte interferon.

It remains to be seen whether the bacteria can be further manipulated to



increase their yield of interferon, currently running at one thousand fold less than that produced from an equivalent culture volume of human lymphocytes. Professor Weissmann is confident that that can be done. He is somewhat more concerned about the potential clinical activity of bacterially produced interferon, since it is unlikely that bacteria can add the carbohydrate chains that are naturally present on the protein molecule. Current indications, however, are that this will not matter clinically.

If the carbohydrate groups do turn out to improve clinical activity, genetic engineering will probably be a less profitable route to interferon than the alternative approach of cell culture, which is already producing enough interferon for some clinical trials. For example, the Wellcome Research Laboratory in Beckenham, UK already extracts interferon from virus-transformed lymphoblastoid cells in sufficient quantities to satisfy a UK Medical Research

Council sponsored trial in myelomatosis. Many, perhaps dozens, of other laboratories are undertaking a similar approach although some of them, such as Searle Laboratories in High Wycombe, UK, use fibroblasts rather than lymphoblastoid cells.

Biogen is not alone in its genetic engineering approach to interferon. Amongst other industrial concerns, some of them with an undeclared stake in the business, Searle Laboratories and the Dupont Chemical Co (in Wilmington, Delaware) are thought to be particularly close on Biogen's tail. Dupont in collaboration with the California Institute of Technology has recently sequenced one end of interferon. They will now probably synthesise the complementary DNA sequence and use it to fish out bacterial clones that contain interferon genes.

What must already concern Biogen's industrial competitors is the patents that Schering-Plough have applied for on behalf of Biogen in which they hold 15% of the shares. Professor Weissmann says that the patents are connected with the plasmid rather than the process and no one knows whether they will be granted. In any case, most of the eventual profits that accrue from interferon or any other Biogen product will finish up in the US, a fact which does not please Professor Weissmann, himself a shareholder.

It is a great pity, he told *Nature*, that none of the Swiss or German companies that were originally approached was willing to take a major share in Biogen. The more the pity because several eminent European scientists are attached to the company and because its own laboratories are in Geneva. These are already occupied by the first recruits and a director of research is being sought. The interferon work has been carried out during the last 18 months in Professor Weissmann's own laboratory but was paid for by Biogen.

Peter Newmark

United States

Registration proposed for private DNA research

A bill that would require companies to register details of their research and production activities involving the use of recombinant DNA techniques is to be introduced into the US Senate by Senator Adlai Stevenson, chairman of the Science, Technology and Space Subcommittee.

The proposed bill, which Senator Stevenson refers to as a "reasonable alternative" to comprehensive regulation, would cover individuals and institutions not presently required to observe the National Institutes of Health's safety guidelines. The Secretary of Health,

Education and Welfare would have to be notified of all research, development and production activities using these techniques — including the source of the DNA, the type of hostvector system used, and the volume of growth media — with a civil penalty for non-compliance.

The bill also includes procedures to guarantee the protection of trade secrets. And to provide an opportunity to evaluate claims of voluntary compliance with the NIH guidelines, it would also require the secretary of HEW to report annually to Congress on the adequacy of safety

precautions reported to have been taken.

Senator Stevenson is proposing to schedule hearings within the near future on the status of current regulation and development of recombinant DNA research. How far the bill goes, however, will depend partly on the support that it gets from Senator Edward Kennedy's Human Resources Subcommittee, which has jurisdiction over medical research — and with the presidential campaign now in full swing, the subcommittee is not expected to give the bill high priority.

David Dickson

United Kingdom

Biotechnology report urges £10 million programme “to match competitors”

A MAJOR report on biotechnology in Britain — not yet published but made available to *Nature* — calls for a rapid increase in investment to build a competitive industry. It says the “customer-contractor” principle should be scrapped for biotechnology, where the border-lines between basic and applied research are grey. The report is a first draft — though unlikely to be substantially altered — from a seven-man working party set up early last year under Dr Alfred Spinks, a former research director of Imperial Chemical Industries. **Robert Walgate** reviews its hard-hitting and interventionist recommendations.

THE working party was drawn from the Royal Society, the Advisory Board for the Research Councils and the Advisory Committee for Applied Research and Development. It was set up under the previous Labour administration; during its deliberations a Conservative government, committed to non-interference in industrial affairs, has taken office, but this has not restrained its far-reaching proposals.

It says the whole foundation of biotechnological research in Britain should be changed:

“Since 1972, the UK has unlike other countries adopted a very rigorous customer-contractor principle for all its applied R&D funded by government. This approach is well-adapted to short-term or tactical applied work with clear objectives which can be defined by a single customer.

But biotechnology epitomises a field in which scientific knowledge and technical expertise are evolving rapidly, where definite customers have yet to develop in any number; where the research of government departments is divided; where the research will be of interest to a wide range of private industry, public services, etc.; and where the principle need for growth is in strategic applied research much of which will not yield results of immediate commercial benefit for perhaps a decade.

“For such a field the customer-contractor principle has serious shortcomings, and may exacerbate the situation by emphasising an arbitrary and unreal division between fundamental and applied research.” Nevertheless the ultimate objective should be to make British industry competitive with the best of its foreign rivals.

Among its recommendations are:

- That the research councils and the ABRC should set up a Joint Committee for Biotechnology to develop a coherent programme of biotechnology research and to coordinate spending of not less than £3million annually (including existing research council initiatives). The Joint Committee would aim to set up new projects in universities and elsewhere, and “should encourage collaboration between experimenters of different disciplines, in different departments and institutions”. Applied biologists currently working in medical or agricultural studies should be encouraged to take up biotechnology. A condition might be applied to applicants for grants that they should show evidence of industrial interest in their project.

- That an Interdepartmental Steering Group should be sent up to coordinate the actions of ministries and develop a programme of industrial R&D. The Department of Industry might take a lead in this, setting up cooperative programmes with industry (funded 50:50), research associations, government research establishments, universities, and inventors amounting to £2.5million annually (including existing projects).

- The Steering Group should consider setting up a Support Unit, like the Department of Energy’s Energy Technology Support Unit, to do desk studies and build up a list of potential projects for which applications would be sought.

- The Joint Committee and Steering Group should have overlapping membership to avoid a division between basic

and applied work, and in any case should review the position in 4-5 years’ time.

- There should be no new establishments specifically to pursue research in biotechnology; these can stagnate and exacerbate interdisciplinary divisions. But the Department of Industry should co-sponsor the Centre for Applied Microbiological Research at Porton Down (it is presently sponsored by the Public Health Laboratory Service); CAMR expertise could be well-applied to biotechnology.

- The University Grants Committee and the Committee of Vice-Chancellors and Principals should support the expansion of a limited number of centres of excellence in biotechnology from the best existing in universities. A minimum of 20 new teaching and research post should be established over the next five years with a capital investment of around £2million to provide additional laboratory facilities.

- Urgent provision must be made for training a work-force to match the expected growth of biotechnology; greater interaction should be encouraged between departments and undergraduate courses in the biological, chemical and engineering sciences.

- The Science Research Council’s Cooperative Awards In Science and Engineering, which funds research students in joint projects between university and industry, should be extended to the Agricultural and Medical Research Councils; and there should be analogous post-doctoral awards.

“Government should not impede the rapid development of biotechnology by inappropriate or unfounded concern for existing industries or the conjectural hazards apparently presented by some aspects of the new technology.”



Dr Spinks: he chaired the working party.

- The National Research Development Corporation, one of whose roles is to patent and license appropriate inventions made in universities, "should play a more entrepreneurial role in this area, where inventions are often not readily patentable. The NRDC should expand its staff in biotechnology and review existing financial incentive for academic inventors. The NRDC should be at least as concerned with the development of new business as with obtaining and licensing industrial property.

- The NRDC should continue to pursue with the Patents Office the patenting of microorganisms and press for arrangements, as in the US, which do not require the microorganism to be made available until the patent is granted.

- As new research defines the hazards of genetic manipulation, the UK in consultation with others from abroad should modify regulations appropriately. The Genetic Manipulation Advisory Group and the Health and Safety Executive should continue to reduce constraints while maintaining an adequate level of safety and introduce procedures to ease industrial application.

- The National Enterprise Board and the NRDC should investigate the use of public funds to set up a research oriented biotechnology company (akin to the American Biogen and Cetus). £2million a year for five years should be sufficient to establish if it could be profitable, the report suggests.

- The Ministry of Agriculture, Fisheries and Food and the Department of Industry should jointly investigate the use of agricultural products as feedstocks for

biotechnology.

- The Department of Health and Social Security should adjust its purchasing policy to encourage small biotechnological firms.

- The Department of the Environment together with the Department of Industry and the Natural Environment Research Council should consider the use of biotechnology for waste disposal and materials recovery.

- If the European Economic Community decides to pursue its research programme in biotechnology, the UK should seek maximum return from it.

- Government should seek fiscal regulation within the EEC which will allow the use of agricultural feedstocks for industrial biotechnology "and not impede the introduction of new processes based on biotechnology". The report here criticises "the strong lobbies of established industry and agriculture which tend to exert a disproportionate weight in resisting the cheaper and better products of new technology, particularly where these threaten to perturb existing patterns of agriculture". There should be assurances that processes "will not invite penal levies... We are concerned that this appears to be the present case."

- Addressing itself to the European Community, the report recommends that "all member states should consider the value of biotechnology in transforming agricultural surpluses, and should seek to amend the Common Agricultural Policy legislation that threatens this end". Further, the EEC directive on the control of genetic manipulation is outdated and should be "reconsidered".

- The report "supports" the SRC in a £1.5million capital, £1million annual expenditure on biotechnology; commends the ARC's initiatives in genetic manipulation, and makes a mild and indirect criticism of the MRC: "The MRC and research councils should review their roles in relation to industrial development based on biological processes so that their staff are aware of the desirability of creating, where possible, a national return for their efforts through industrial development."

Despite the case of cephalosporins (antibiotics emerging from MRC research which are now the NRDC's single greatest revenue earner) "the take-up of discoveries by UK industry is not encouraging". Extra facilities might be provided to researchers for applied work in their own laboratories, or a special development unit might be established. However, the MRC was not set up with industrial development as an objective, and any moves along these lines would require extra resources.

National Collection of Yeast Cultures threatened

THE UK National Collection of Yeast Cultures, one of the most used in Europe, faces closure at the end of this year — unless the Agricultural Research Council can find a meagre £20,000 per annum to support it. "I don't see how biotechnology can carry on without a repository of cultures from which one can select strains" said a yeast microbiologist last week.

The collection has been looking for a home since early 1979, when one of its present co-sponsors, the Brewing Research Foundation, announced that it would withdraw its one-third contribution to the costs of the collection from January 1981. The major sponsor, the Ministry of Agriculture, Fisheries and Food, also decided to withdraw at the same date. The collection must also move from its present location at the BRF, in Nutfield, Surrey.

All would be well, say microbiologists, if there was some firm promise of new funds and a new place for the collection. But despite detailed recommendations to the Agricultural Research Council — who it is widely believed should support the collection — from the UK Federation for Culture Collections, no decision has been reached on either location or funding.

"Individual collections are not sufficient" said one microbiologist. "We keep our own small collections but we do not have time, competence or staff to do routine testing or distribute samples. It would be very serious if we sent the wrong organism".

The UKFCC has recommended that the collection and its four staff be established at the ARC Food Research Institute in Norwich, on a site where the John Innes Institute and the University of East Anglia are already established.

MAFF has placed the problem firmly with the Advisory Board for the Research Council's, which in turn has passed the problem to the ARC. But it would appear that the ARC is not prepared to fund the collection unless it receives extra finance.

Barbara Kirsop, the curator of the collection, told *Nature* that the collection has been in financial difficulty for four years. A number of agencies picked up the bill for short periods in the past, including the ARC which paid 50% of the bill for one year in 1976. "The BRF" said Kirsop "no longer wishes to support the collection because its emphasis has changed over the past ten years. People are using yeasts a lot more and we have constructed a very physiological collection which means we have a great number of yeasts with many interesting and useful properties". The collection holds 10-15 patented strains among a collection of 2000 varieties. "We need to start moving the collection in June, if we are to be out by December," says Kirsop. □

NEWS IN BRIEF

Europe's biotechnology programme one step nearer

THE European Commission has approved the £16 million, five-year research programme on biotechnology described recently in *Nature* (10 January, page 125). But the programme still has one hurdle to jump before it can be implemented: the Council of Ministers, where the nine EEC member states take final control over Commission proposals.

The Council will take advice from CREST, the Commission's official joint consultative committee for science and technology, in February; and from the European Parliament early in March. If there were no national objections, the programme could be approved in April.

IAEA to hold closed nuclear safety conference

THE International Atomic Energy Agency in Vienna has organised an international conference on current nuclear plant safety issues to be held in Stockholm 20-24 October 1980. Reinhard Schmidt, scientific coordinator of the conference describes it as being within "the framework of those who want and need nuclear power". The conference hopes to address four main issues: assessment of current safety issues, improvements in safety, information exchanges, and development of increased national cooperation. Plenary sessions will be held with "exclusively invited papers" and conference participation "whether or not a paper will be presented must be through designation by the government of a member state of the IAEA". The meeting is expected to be open to the Press although a final decision has not been taken on this yet.

Academy report calls for orderly energy transition

A panel of the US National Academy of Sciences stated last week that both a significant increase in energy efficiency, and the development of new sources of energy — including the fast breeder nuclear reactor — were necessary to make an "orderly and smooth" transition to a coming era of energy scarcity.

The report, prepared by an *ad hoc* Committee on Nuclear and Alternative Energy Systems (CONAES) under a commission from the Department of Energy, emphasised in particular the importance of energy demand considerations in planning future energy supplies.

In a covering letter submitting the report to the department, the two co-chairman, Dr Harvey Brooks of Harvard University

and Dr Edward L. Ginzton of Varian Associated, emphasise the importance of remembering that the energy problem does not arise from an overall physical shortage of resources.

"The problem is effecting a socially acceptable and smooth transition from gradually depleting resources of oil and natural gas to new technologies whose potential are not now fully developed and whose costs are generally unpredictable", they say.

The report also warns that current uncertainty about the problem associated with the development of nuclear fusion as a source of power means that it cannot be relied upon as a source of energy, at least not until well into the next century.

New head for US Atmospheric Research Center

DR Robert M. White, for many years head of the US National Oceanic and Atmospheric Administration and its predecessor organisations, has been appointed president of the University Corporation for Atmospheric Research, responsible for operating the National Center for Atmospheric Research in Boulder, Colorado, under the sponsorship of the National Science Foundation.

Dr White, who will succeed Dr Francis Bretherton as the full-time chief executive of UCAR, is currently administrator of the National Academy of Sciences' National Research Council. He has served as the US permanent representative to the World Meteorological Organisation since 1963, and was chairman of the WMO's world climate conference, held in Geneva in February last year. Dr Bretherton has relinquished his position in order to return full-time to scientific research.

Defector details NATO first nuclear strike plans

THE former senior secretary to the director of war games at NATO's Brussels headquarters said last week that NATO leadership planned to make first use of nuclear weapons in the event of a conflict with Eastern Europe. Ursel Lorenzen, who defected to East Germany last year, told a press conference in Berlin that NATO also had detailed plans to start a war by provoking major incidents such as blocking Eastern European shipping lanes or activating remote controlled mines in international waters. Ms Lorenzen, aged 41, said that for 11 years she had watched the planning and development of strategies to start a war with a rapid nuclear strike against East European nuclear targets. "I

was present countless times when high NATO officials and military men confirmed that NATO most certainly in every case would use atomic weapons first" she said. In addition she said that NATO carried out occupation and invasion exercises under simulated Russian winter conditions and that NATO officials had told her that they were drawing on the World War II experience of the Germans. Small NATO member states such as Norway and Denmark who objected to the nuclear strike plans were overruled by the bigger countries, she said.

French police break up researchers protest

A 24 hour occupation of the headquarters of the French national institute of health and medical research (INSERM) by medical researchers was broken up by police at the request of the directors of INSERM last week. Four hundred members of the trade unions representing French medical researchers occupied the headquarters to protest about the government's changes in the status of researchers in INSERM and the Centre Nationale de la Recherche Scientifique (CNRS). The unions called recourse to police action an "illustration of the determination of the institute's director as well as the minister of health and the secretary of state for research not to negotiate". The sensitive points in the new statutes include introduction of a new four year probationary period, a lowering of the maximum age for entry into the profession to 27 years old, and forced mobility at the discretion of research directors.

United Nations University delegation visits USSR

A top level delegation from the UN university visited the USSR from 6 to 11 January at the invitation of Academician D. M. Gvishiani, vice-Chairman of the State Committee of the USSR for Science and Technology. The purpose of the visit was to establish increased cooperation between the USSR and the university. Talks were described as "productive" but no formal agreements have been finalised. A similar visit to China last May resulted in the creation of two joint research projects in areas of the university's strength — one a study of land-water interaction in the Pearl River basin in China, the other a remote sensing project in the Netherlands — as well as the placing of Chinese scientists for limited periods in western research institutes and universities. "I think we can expect a similar outcome of this visit" a UN official said.

FEATURES

Middle East brain drain switches back from the West

The brain drain of scientists from the poorer countries of the Middle East has switched from the industrialised West to the oil-rich countries of the region. And some countries are running successful schemes to attract expatriate scientists back.

Ziauddin Sardar reports

THE pattern of the brain drain from the Muslim countries of the Middle East and Pakistan has changed considerably in the past few years. In the sixties and early seventies the flow of scientific and technical expertise was almost exclusively from the Muslim world to the developed countries. As science could not be "consumed" at home or bring adequate financial rewards, the only satisfying career for the up and coming Muslim scientists was in the universities and industries of the West.

This South-North flow of scientific manpower has now given way to a South-South flow: scientists from Muslim countries such as Pakistan are now going to the Middle East. Moreover, many Muslim scientists who had settled in the West or who had trained there are persuaded by the prospect of greater prestige or simply better salaries to return home. Those who have just trained are also finding difficulty in settling in the West because of tighter immigration laws.

The changing pattern of the brain drain is welcomed by scientists and science policy makers in the countries involved. Although no figures are available, the brain drain from Pakistan to the oil-rich states of the Middle East, for example, is thought to be considerably higher than the drain to the West has ever been. But, according to Ghulam Mirza Galani, Secretary of Pakistan's Science Council, "this does not worry Pakistan for two reasons. Firstly, it is not a permanent brain drain. Pakistani scientists working in the Middle East always return home after four or five years, once they have made their money. This contrasts sharply with our brain drain to the West where Pakistani expatriates have tended to settle down. Secondly, while working in the Middle East Pakistani scientists send home much needed foreign exchange so they are not completely cut off from us".

In certain Arab countries the brain drain to oil-rich countries is actually encouraged by the government. Egypt, for example, educates its graduates for the technical-manpower market of oil-rich states. Most

Egyptian universities have courses on petrochemicals, desalination and high technology agriculture. Some of these special education programmes are recognised only in the Arab world and a few particular qualifications are only recognised in specific countries. Certain Egyptian medical degrees and doctorates, for example, are valid outside only in Saudi Arabia.

Syria, unlike Egypt, faces an acute shortage of scientific manpower, but it

manages to provide facilities for its scientists who wish to work in other Arab states. "Once it used to be the United States, West Germany and France", says Dr Asaad Lutfi, Vice-Rector for Scientific Affairs, University of Damascus, "but now our brightest scientists wish to go to Saudi Arabia, Libya and the Gulf."

"But we do not feel bad about this. It is our responsibility to help other Arab states. We see this as our scientists making an important contribution to the development of the region." Scientists who wish to work in other Arab states are granted up to 4 years' leave of absence; in many cases the government helps them find employment in the country of their choice. According to Dr Lutfi over one-third of Syria's scientific manpower is using the scheme.

As exporters of manpower, both Egypt and Syria have an advantage over the other main manpower exporter to the Arab region — Pakistan — being Arabic-speaking. Egyptian scientists seem to favour Saudi Arabia and the Gulf whereas

LEBANESE agronomists testing soil humidity: until four or five years ago, the Lebanon was a major training ground for scientists in the Middle East. The American University in Beirut, in particular, trained many scientists for the region.



the Syrians prefer Algeria where the language of science is being changed from French to Arabic.

Rich Arab countries are also making efforts to attract the pool of Muslim scientists and technologists working in the West.

Attracting the expatriates home

For example, Libya has set up the Tripoli-based Arab Development Institute — which despite its name is an organisation devoted to scientific research in fields that range from biology to solar energy — and provides it with almost unlimited resources. Although it employs only 70 scientists, it is particularly attractive to Muslim scientists working in the West because of the high salaries — a young PhD can earn \$2000 per month — and the excellent facilities. According to Dr Kamel Al Din Hassan, head of solar energy research, “when a project is approved, the scientists simply go ahead and spend what they have to. Research is what counts, budget limitations just do not apply.” Publication is also guaranteed in the ADI’s own quarterly research journal. The institute now runs recruiting offices in Rabat, London and Washington and is planning opening offices in every Arab capital. The Libyans consider it an outstanding success.

But not all Muslim countries have access to endless financial resources. Turkey, for example, has had to seek alternative ways of attracting back its scientists. In 1977, the Turkish government, with the help of the UN Development Programme, initiated the Project for Transfer of Know-How Through Expatriate Nationals (TOKTEN) with the aim of securing the expertise gained abroad by the many expatriate Turkish scientists and technologists. After two years in operation the TOKTEN project has proved to be ingenious and highly workable.

The main objective of TOKTEN is for specialists of Turkish origin now resident in the West to provide specific technical help. It gives preference to scientists of international standing whose services are obtained for short assignments of one week to a maximum of three months. The visiting scientist transmits his knowledge through training seminars, consultations, reports and proposals on policy issues, and other advisory services. The UNDP meets the travel costs, and living expenses, but pays no salary; the host institution pays an honorarium.

This method of consultancy by expatriates has proved far superior to traditional methods of seeking technical assistance. According to Professor Hakki Oranc, Assistant Secretary General of the Scientific and Technical Research Council of Turkey (TUBITAK), “in general a rich return on the time invested by the consultant has been achieved. There is no

need for a period of adaptation, adjustment, acquaintance of the consultant with local conditions, or for the establishment of his credibility. His cultural affinity to Turkey improves his acceptance and effectiveness”.

There are further reasons why TOKTEN has proved to be more successful than most multi-lateral technical cooperation programmes. The Turkish expatriates provide a faster response to an institution’s problem than is possible through traditional technical cooperation. Sometimes the character and confidentiality of the desired advice requires a privileged relationship which is easier to ensure with a trusted expatriate. Often the full value of a training programme or seminar can be ensured only if it is conducted in Turkish.

Quite a number of expatriate Turkish scientists have become specialists in solar energy research. Some have been brought under the TOKTEN scheme to Turkey for various lengths of time bringing the latest know-how. This should make a significant contribution to the success of a proposed regional centre for the Mediterranean on alternative sources of energy, which the UNDP is expected to support in southern Turkey. The government also hopes to seek the help of an equally large group of expatriate nuclear scientists in its attempt to make Turkey self-sufficient in nuclear power by the turn of the century (see *Nature* 282, 670; 1979).

“The TOKTEN project has shown that Turkish expatriate scientists are motivated by a desire to help their homeland and not by financial gain”, says Professor Oranc. “Of course, we hope that some of them will get so involved with their assignments that they will prefer to work in Turkey. But until such time, scientific and technical gains to Turkey from their efforts are proving to be much higher than one would expect from traditional technical assistance provided at much higher cost”, he says.

Second-class citizens

Despite considerable economic gains, Muslim scientists working in the Middle East complain bitterly about the working conditions. The most common complaint is that they are treated as second-class citizens. One Saudi scientist, Dr Raghib El Naggar, an Egyptian says: “expatriate scientists working in Saudi Arabia, the Gulf and Libya are treated viciously. They have no rights whatsoever and virtually no prospects for promotion. No wonder then that most of them leave as soon as they have gained their financial goals”. Expatriate scientists in the Middle East are also facing the same problems of corruption and bureaucracy that they are running from in their native countries. “Many expatriates believe that if they have to fight corruption and bureaucracy, they would rather stage the battle back home. There they have a higher probability of success”.

Institut

THE Institut Pasteur is a monument to great men. Pasteur himself is entombed there, in a glorious mosaic sepulchre; images of Faith, Hope, Charity — and Science — gaze down on his casket, and chickens, cows and silkworms decorate the walls. His spirit, and that of the more recently deceased director, Jaques Monod, still inhabit the place.

One of Pasteur’s passions was that the new biology — his discovery of the role of microorganisms in disease — should be applied to medicine and agriculture. Biology was not an end in itself but existed to help people, industry, and France. There was a certain practical socialism to his vision, and it remains with the Institut Pasteur today.

Now, François Jacob believes, there is a dual conflict with industry. People in the universities have contempt for applied work, “and industry considers that the scientists are leftist, impossible people”.

The Pasteur has a factory that contributes 20% of the funds, with 50% coming from government and 30% from legacies and donations, but negotiations are going on to increase the proportion from manufacture by involving private firms.

Pasteur himself, said Jacob, was able to do all his industrial work in the laboratory, on the bench — “and this was still possible up to the last war; but after the war it didn’t work like that at all, and the Institut’s funds began to decline”.

And despite the Pasteur’s history “even here it is not easy to put the people in the factory and the people in the research laboratory together”. There is financial encouragement to do applied work, but few Pasteur researchers take it up.

The division is also present in biotechnology. Jacob has recently co-authored a report on the subject with François Gros, Director of the Pasteur, and Pierre Royer, President of the administrative council; they recommend that France’s principle research funding body, the DGRST (Directorate Generale pour la Recherche Scientifique et Technique), should control any initiative that France might take.

“We have a secretary of state for science, Pierre Aigrain, and of course we have a minister for industry — and there is already a debate between them over who does what and who gets the money”. The DGRST is under Aigrain, and “it is better that they make moves towards industry than industry towards science. But the tendency here is to give the whole thing to industry”.

But new firms, such as Biogen and Genentech in the US “are interesting: probably much of this biotechnology is small business anyway, and the small firms may be the best way to put things together . . . In our report we suggested it would be

Pasteur seeks out industrial support

François Jacob wants industry's money for fundamental biology — but not its control. A Nobel laureate for his work on the *lac* operon (he shared the prize with Jaques Monod), he is head of the cellular genetics unit in the department of molecular biology, Institut Pasteur in Paris. He spoke with **Robert Walgate**

better, to push biotechnology with small enterprises, like wine making and so on".

In the laboratory, secrecy and confidentiality were going to be a problem, but the secrecy "will really be extremely limited . . . it will concern what people are actually doing; it has nothing much to do with the basic principles. It may be some trick to get some particular messenger, or something like that; it is unpleasant, because secrecy is always unpleasant, but I don't think it is a severe problem — in this case anyway."

"To know that these guys are doing insulin is not a very big secret; what is important is to know how it works."

"What is worse is that industry is going to control a lot of basic research. Because if, as usual, a researcher has difficulty in getting money, and if he can get money only in what interests industry, industry is going to channel research in its own directions. I think this is more injurious than secrecy."

How could this be dealt with? "Well, the channelled industry money must be kept down to a certain fraction of the research budget. I have nothing against giving *free* money!"

But this would be difficult to police; "and that's exactly what we are fighting with in the Institut Pasteur. We have had troubles with industry since the war; and now there is an attempt to cooperate more narrowly with some big firms."

"We are trying to make arrangements that are not injurious to the Pasteur. The French pharmaceutical industry has done very little for basic research — except for two or three firms. Most of them have done only very narrowly directed, applied research."

"It's always unpleasant to have to negotiate with these people. What they would like to do is to give money to the Pasteur as a function of contracts; but we want the main part of the money to be completely free for basic research."

The problem is the Institut's factory, which is "either too big or too small". The factory has to make vaccines "which are used only in the case of epidemics — to avoid situations like Brazil some years ago when a lot of people died of a meningococcal infection. The big drug companies are not interested in that. We are making stocks of these things here but there is no profit in them."

"Profit politics will not go on forever — there will be a time when you have to stop this nonsense — but probably the big drug

companies cannot do it."

Is there a socialist ethic in the Pasteur? "Yes, that was the idea of Pasteur himself, and Monod tried to pick it up." The mood continues very strongly, "and that's exactly the difficulty of our negotiations with industry".

In the recent report on biotechnology, Jacob wrote the last and most philosophical chapter, on the possible interactions between biology and society in the next 20 years. "The problem was the following. We were asked to produce predictions of the unpredictable . . . and the most interesting thing in science is precisely what is unpredictable."

"What kind of thing could you predict? The first thing I took was the pill, and then the ability to choose a baby's sex, which would not have much impact in Western Europe."

He also predicted brain drugs: two scenarios of psychoactive drugs which would be produced in large quantities through molecular cloning techniques; in one, they would be used by governments as warfare or social control agents; in the other, as a more advanced kind of pill-popping, hyper-valium, opiates of the masses: Aldous Huxley's *Brave New World*.

"You take this seriously or not seriously as you wish; personally, though, I do not take it seriously."



Francis Jacob — carrying on the traditions of Pasteur.

More serious was the transformation of certain ideologies from biology into political beliefs like social Darwinism or eugenics.

Jacob allows himself one remark however: "the genetic diversity which creates the richness of animal and vegetable species also creates that of the human species," he writes in the report.

"A population composed of individuals genetically too alike would find itself at the mercy of an accident — an epidemic or a sudden shift in the conditions of life. All efforts towards a uniformity in individual biological characteristics, be it towards an 'improvement' of the race through eugenics, or towards the increase of an aptitude for mathematics or for running, would be biologically suicidal and socially absurd".

Scientists should discuss their work, with as little secrecy as possible; they should point out what is possible in five or ten years, even if they think it will not come to pass; and then there have to be enough people who are not scientists making the effort to understand.

"These questions should not be restricted to scientists and/or politicians. Reasonable people from outside must come in — but then they have to make the effort, and the scientists have to make the effort, to talk to each other and understand what is going on."

Professor Jacob proposes that the Academie des Sciences should coordinate such discussions. "I'm not sure they will, but they should". Or there could be a European forum: the European Molecular Biological Organisation could do something, for example.

However, Jacob is not hopeful that France will join in with the European programme on biotechnology presently under discussion in Brussels (*Nature* 10 January p 125).

"The French attitude will probably be nationalistic. But that is just stupid, because I think Western Europe is just going to be eaten in the next 50 years. I'm very pessimistic — aren't you? I just don't see how one can be optimistic. There are many reasons but especially because of this tremendous differential increase in population between Europe and the rest of the world . . . And then there will be the problem of food; you will have some fancy food technology in the West while the rest starve . . . it's a tremendous mess".

François Jacob, who was a Spitfire pilot in the war, and had to get up once or twice during our interview to ease his shrapnel wounds, told me "I personally am very much a European and have always been so since the war; but it's a fact that nationalism is on the increase, not more in England than in France. The whole thing is just incredible". □

CORRESPONDENCE

Clerk-Maxwell centenary

SIR, — It is not my intention to spoil the effect of the perfect tribute paid by Professor Domb in *Nature* (15 November, page 235) to Maxwell on the occasion of the centenary of his death. But could I be permitted to make a minor point and to mention Maxwell's ideas about the principle of relativity of motion?

Professor Domb's remark that the true quality of Maxwell's genius became apparent only with the development of twentieth century physics, might lead some to believe that his genius was lost on his immediate successors in the nineteenth century. Far from it. Perhaps, Boltzmann gave the most eloquent praise to Maxwell's equations (of electro-magnetism). On reading these equations Boltzmann exclaimed, "War es ein Gott, der diese Zeichen Schrieb?" (Was it a God, who wrote these signs?) (*Mensch, Physiker, Philosoph.*) Berlin 1957, page 31).

The word "relativity" was first coined by Samuel Coleridge in the year 1834 but he used it in a philosophical sense. It appears that Maxwell was the first ever to use the word relativity with reference to the doctrine of relativity of motion, and this not only for mechanical but also for electromagnetic phenomena. True, he suggested experiments to determine the putative motion of the Earth relative to the "ether" and in fact made an experiment himself to measure this motion, using a rotating spectroscopic prism and partially silvered mirrors but got a negative result (*Phil. Trans.* 1868, page 532). Therefore Maxwell was not sure whether motion relative to the ether could be observed, but he ultimately moved to a firm belief in the relativity of all phenomena.

It is not widely known that in *A Treatise on Electricity and Magnetism* published in the year 1873, Maxwell devoted two articles, to the modifications of the equations of "electromotive intensity" when referred to moving axes. Using a method which is questionable, he yet came to the conclusion that "the electromotive intensity (in the moving system) is expressed by a formula of the same type", and added, "In all phenomena, therefore, relating to closed circuits and the currents in them, it is indifferent whether the axes to which we refer the system be at rest or in motion". This was more than an intimation of things to come.

Four years later in the year 1877, Maxwell published the book *Matter and Motion*, in which he used the word 'relativity' and affirmed, "Our whole progress up to this point may be described as a gradual development of the doctrine of relativity of all physical phenomena." Obviously he expected that the principle would be found to be universally valid. Penetrating deep into the future he proclaimed, "There are no landmarks in space . . . we have no log which we can cast to take a dead reckoning by . . . we may compute our motion with respect to our neighbouring bodies, but we do not know how these may be moving in space."

However, he thought as Newton before him did, that the doctrine of relativity broke down in the case of rotational motion, saying, "But though it is impossible to determine the absolute velocity of a body in space, it is possible to determine whether the direction of a line in a material system is constant or variable. For instance, it is possible by observations made on the Earth alone, without reference to the heavenly bodies, to determine whether the Earth is rotating or not". Maxwell then quoted Newton's bucket-experiment, carrying the bucket in thought to the North Pole. However, finding the calculated depression of the water-surface in the bucket

too small for measurement, Maxwell remarked that the most satisfactory experimental proof was Foucault's pendulum, the rotation of which (except at the equator) establishes the absolute rotation of the Earth, although the stars and the pendulum are in no visible manner connected.

Probably Poincaré, who developed these ideas soon after, and used the phrase "Le principe de relativité," followed in Maxwell's footsteps — and, of course, travelled farther. Otherwise, how could we explain the exact transliteration of "relativity" into "relativité"?

Yours faithfully,
G. H. KESWANI

Tata Institute of Fundamental Research,
Bombay, India

Dioxin detection

SIR, — *Nature* is too important a scientific forum to leave unchallenged the inaccuracies contained in the report (8 March, 1979, page 109) of my comments about comparative laboratory measurements of 2,3,7,8-tetrachlorodibenzo-paradioxin (TCDD or dioxin).

Alastair Hay failed to provide any context to my comments and they appear to be an admission that Dow's analytical capabilities are poor in evaluating the dioxin content of environmental samples. This, of course, is not true. Dow chemists are now confident that the 2,3,7,8-TCDD molecule can be isolated from its isomers and detected at the 5 parts per trillion (10^{12}) level in environmental samples.

With that as background, let me move to the incident reported in your 8 March issue — the EPA study to evaluate the ability of the Agency's Mississippi Test Facility to "spike" and clean up samples of beef fat and human milk; and the determination of abilities of various laboratories (University of Nebraska, Harvard University, EPA's PTSEL Laboratory at Research Triangle Park, Wright State University and Dow Chemical) to measure TCDD in final extracts.

Beef fat was spiked and extracted by EPA Mississippi Test Facility using an acid/base procedure. Identical samples were shipped to Lab A and Lab B. An equivalent set was shipped to Lab C at a different time. Other extracts of beef fat were shipped to Lab D and Lab E. All the laboratories made measurements of TCDD. Of the total 20 unspiked samples measured, 10 gave false positive results. The 15 samples spiked with 0.5 to 9 ppt TCDD showed seven false positive results and zero false negative results. Lab C did not show false results below 9 ppt because its detection limit was too high to make measurements. The 94 samples spiked with 9-81 ppt TCDD showed two false negative results and one false positive.

Spiked beef fat was shipped to Lab D to evaluate its neutral extraction procedure for TCDD. These extracts were examined by Lab D and Lab C. Of six extracts of unspiked samples, one showed false positive results. The 18 spiked below 9 ppt showed six false positive results and zero false negative results. Above 9 ppt, two of 38 extracts gave false positive results and one false negative.

Extracts of human milk were shipped to Lab A and Lab B. Of 12 unspiked extracts, 11 gave false positive results. Of 12 extracts spiked with 0.5 to 9 ppt TCDD, two gave false positive results and three false negative. The 53 extracts spiked above 9 ppt TCDD gave no false positive results and no false negative results.

So the collaborators correctly concluded that the method gave acceptable results above 9 ppt but none of the laboratories was able to produce satisfactory data below 9 ppt.

These facts may be accounted for by any or

all of the following reasons: the clean-up may be unsuitable for the particular mass spectrometer being used; the extracts may have stood too long before measurement; or the mass spectrometer may not have been performing properly.

The results obtained in this study in no way compromise results obtained by the various laboratories in other studies in which they did their own extractions and clean up.

Yours faithfully,
WARREN B. CRUMMETT

Dow Chemical, Michigan, US

Closed universities better than "sophistry centres"

SIR, — It was obvious that somebody would point out that the 1960s expansion of universities has overstretched resources, and now the words have come, from the mouth of Lord Todd and others. Since the resources derive from the taxpayers, one has only to persuade them that what Lord Todd says is true and it will become so — universities have little else in the way of income, at least as far as scientific research is concerned.

But is the answer to encourage low-caste universities to give up research? Lord Todd is surely as aware as anybody else how research stimulates — even positively goads — teachers into keeping ahead, delving in hidden places and generally searching out the truth. Who benefits most? — the students of course. Without research, no chemistry department is any good at all, and the outcome of Lord Todd's suggestion will be to create not teaching centres but sophistry centres.

If things are so bad we should not be aghast at the idea of closing down a few universities completely; it would be better than trying to keep alive an impotent, wishy-washy, limping sort of place in every university town.

Yours faithfully,
ROY BALLARD

Norwich, UK

Iranian terrorists

SIR, — The cynical apology of the Iranian terrorists in *Nature* (29 November, page 439) reminds one of Geoffrey Dawson's infamous editorial in *The Times* of 7 September, 1938. Brutal terror will always appeal to a certain kind of intelligentsia. Let them have their fun, but please spare us the hypocrisy of preaching to the victims and not to the henchmen. Europe no longer has a Churchill to redeem a Munich.

Yours faithfully,
S. V. VAECK SC

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Islamic science

SIR, — I appreciate that the Islamic world has lived under western colonisation and oppression, particularly economic oppression, for centuries. However, I found that the article 'A revival for Islam, a boost for science?' (22 November, page 354) has a religious bias and is highly racist and chauvinistic. I do not recall any great scientist from Galileo to Einstein mentioning a religious science.

A quotation on page 354 states: "... The new civilisation of Islam will be capable of boosting scientific activity and scientific knowledge to heights and achievements unknown to man before". Surely boosting would have been more appropriate round. However, the simplest, scientific question that the statement begs is: why?

Your faithfully,
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NEWS AND VIEWS

Dust grain orbits in the Solar System

from David W. Hughes

LONG gone are the days when interplanetary dust grains were thought to move in simple elliptical orbits around the Sun. Today three factors in addition to the gravitational field have to be taken into account in determining their orbits.

First, there is a force acting on the grain simply because it is being illuminated by solar electromagnetic radiation. This force contains a radiation pressure term which is related to the ratio between the amount of solar photon momentum intercepted by the grain and the grain mass. For certain particle sizes and materials this radiation pressure force can even exceed the solar gravitational attraction and the particle can be 'blown' out of the Solar System.

Energy absorbed from the solar radiation flux is reradiated isotropically in the frame of reference of the particle. In the rest frame of the Sun however, more momentum is radiated in the direction of motion of the particle than in other directions and this results in a dynamic drag and deceleration. This is known as the Poynting–Robertson effect. Due to this the eccentricities of the orbits of large particles slowly decrease and the particles spiral in towards the Sun in a time which is proportional to the product of radius and density.

Another, more subtle radiation drag operates due to the rotation of the Sun. Light from the advancing half is blue-shifted, increasing its momentum. Light from the retreating half is red-shifted and the resulting asymmetry in the flux of radiation momentum produces an additional force on the particle transverse to the radiation direction. This force is only significant when the particle is close to the solar surface.

A spinning particle which takes time to attain thermal equilibrium will have a skewed temperature pattern. In other words it will have an evening hemisphere that is on average slightly warmer than the morning hemisphere. The warmer hemisphere radiates more energy and momentum, and the integrated reaction force will have a transverse component

somewhat analogous to the non-gravitational forces that perturb cometary orbits. This so-called Yarkovsky effect can increase or decrease the semi-major axis of the particle orbit dependent on the spin direction. The spin speed and particle composition are also important. It is only significant, though, for large particles of over a metre in size.

The second factor affecting the particle orbit is similar to the Poynting–Robertson effect but is caused by the absorption and re-emission of solar wind protons.

The relative significance of the effects mentioned above has been reviewed in a recent paper by J.A. Burns, P.L. Lamy and S. Soter in *Icarus* (40, 1; 1979).

The third factor leading to changes in the orbit of a particle is the electromagnetic charge. A force is exerted on a charged particle as it moves in the interplanetary electromagnetic field of the solar wind. This effect has been studied in detail by G.E. Morfill and E. Grün of the Max-Planck-Institut für Kernphysik, Heidelberg in a recent paper in *Planetary and Space Science* (27, 1269; 1979). All dust particles in the heliosphere, a circular region around the Sun extending out to a distance of about 50 AU, have a potential of about 10 V. This constant value is due to the fact that the mechanism causing charging (electron emission induced by solar ultraviolet radiation) and the mechanism responsible for charge decay (electron influx and subsequent recombination) both decrease inversely with the square of the distance from the Sun.

At the Earth's orbit the interplanetary magnetic field B is about 5×10^{-5} gauss. There are strong magnetic fields on the Sun and currents are induced in the solar wind plasma as it moves through them. These currents distort the field and this field is carried away from the Sun just as if it were 'frozen-in' to the moving plasma. The Sun takes 27 days to make one complete turn on its axis and a stream of particles emitted over a period of time from an active area forms a loose spiral in space. The associated magnetic field also has a spiral shape. This magnetic spiral is divided into an even number of sectors and the magnetic field is directed in opposite senses

in neighbouring sectors. The solar magnetic field reverses in polarity every 11 years and also the latitudinal extent of the sectors varies in phase with solar activity.

The force acting on the charged particle (the Lorentz force) is proportional to $R\phi$ where R is the particle radius and ϕ is the electrical potential. It is also proportional to $\mathbf{V} \times \mathbf{B}$, the vector product of the relative velocity between the particle and the solar wind magnetic field B , and that field B . Now, as a particle moves from sector to sector in its journey around the Sun the effect of the perturbing Lorentz forces are in opposite directions. However as the sectors are not exactly equal in size and strength, stochastic magnetic scattering occurs. Morfill and Grün consider the effect of this scattering on the orbital inclination of dust grains. It is strongly size dependent. For particles of 10^{-4} cm radii the upper limit is 24° and as the size increases this limit drops, being 3° for 4×10^{-4} cm radii and 0.6° for 10^{-3} cm radii. This result is of importance because it indicates that the thickness of the zodiacal dust cloud, a thickness which corresponds to a root mean square spread of inclination of the order of 20° , cannot be produced entirely by magnetic scattering but must be a characteristic of the source of the dust particles.

Systematic orbital perturbations can be induced electromagnetically due to the fact that the equatorial plane of the Sun and thus of the solar wind magnetic field is at an inclination of about 7.3° to the ecliptic (the plane of the Earth's orbit). The effectiveness of this perturbation depends on the orbital period of the particle. If this is much greater than the solar 11 yr cycle (that is, orbital radii > 10 AU) it cancels out. If it is much less (orbital radii < 1 AU) then inclination changes occur which tend to pull the orbital plane into the solar magnetic equatorial plane. Particles orbiting inside 0.3 AU may even enter regions of magnetic resonance and this can enhance the orbital changes. However random changes in the sector structure weaken these resonances considerably.

Morfill and Grün make an interesting prediction from their calculations. Particle inclinations are either 'focused' towards

the magnetic equator or 'defocused' during alternate solar cycles. This might be observable as a solar cycle variation in the width or thickness of the zodiacal cloud and should be looked for.

The final problem they consider is that of the inclination of the zodiacal cloud. Recent satellite observations have shown that the cloud is inclined at $3.0 \pm 0.3^\circ$ to the ecliptic, these measurements being taken between 0.3 AU and 1 AU. The two main perturbation forces — gravitation and Lorentz — have symmetry planes inclined at 1.6° and 7.3° to the ecliptic respectively. Jupiter, the main agent responsible for gravitational perturbation, has a very minor effect inside 1 AU. Morfill and Grün conclude that the inclination of the observed plane of symmetry of the zodiacal light is considerably affected by the

interaction of the interplanetary magnetic field on the charged dust particles, even if this field distribution doesn't materially alter the thickness of the cloud.

Snags still exist. The particles contributing most effectively to the zodiacal light are thought to have diameters from 10^{-3} to 10^{-2} cm and the magnetic perturbation is strongly size dependent and is small in this range. Also the orbital distribution of the source must have an influence — an influence that decreases as a function of the lifetime of the particle. Certain short-period comets are the main dust source. The 3° tilt of the zodiacal cloud probably results from a combination of the source inclination distribution and the magnetic perturbation but the relative strengths of these influences still need to be calculated. □

particular heavy chain specificity may give rise to progeny expressing a variety of light chains². Since immunoglobulin is not expressed on the surface of B cells until light chains have been synthesised, this would imply that exposure to antigen plays no part in determining the repertoire, but merely triggers the selective proliferation of cells with an existing repertoire.

Further evidence against later changes in the antigen-binding site can be derived from observations on cultured cells undergoing the ontogenetic changes that follow the initial expression of immunoglobulin. These changes take place in the effector region of the immunoglobulin molecule, coded by seven genes specifying the constant regions of the five classes of immunoglobulin: IgM, IgD, IgG (which has three subclasses), IgA and IgE. All cells initially express IgM, in which the antigen-binding region of the heavy chain is linked to a μ chain. Some may then also express IgD; a smaller number subsequently switch to IgG, and fewer still to IgA and IgE. Antisera against the idiotypes — antigenic determinants on the antigen-binding site — of cultured leukaemic cells, or cells transformed by Epstein-Barr virus, show that they remain unchanged from the pre-B stage through at least one heavy-chain switch, from μ chains to α chains (M.D. Cooper, Alabama University). A possible weakness in this evidence is that it is not clear what determinants anti-idiotypic antisera are recognising. Experiments with monoclonal anti-idiotypes defined with polyclonal sera comprise families of determinants some of which may not be associated with the antigen-binding site (K. Rajewsky, Cologne University; A. Nisonoff, Brandeis University). Thus it is conceivable that examination with batteries of monoclonal antisera would reveal subtle ontogenetic changes not detectable by other means.

Some participants did indeed seriously argue that antigen-binding specificities do change during ontogeny, largely on the basis of experiments by A. Coutinho (Basel Institute for Immunology), who claims that B cell precursors bear idiotypes not associated with immunoglobulin molecules and that antisera against these idiotypes can not only trigger growth and differentiation of the cells but induce the expression of immunoglobulin molecules with different idiotypes. This claim, however aroused considerable controversy.

Signals for antibody production

That there is more than one receptor through which B cells can be induced to grow and differentiate, and that antigen is only one of the signals for these processes is no longer seriously disputed. The receptor through which antigen acts on B cells is immunoglobulin expressed on the cell

The life of a B lymphocyte

by Miranda Robertson

THE ontogeny of the B lymphocyte is unique in that it is marked by a series of changes in gene expression which involve chromosomal rearrangements reflected in a single molecule: the immunoglobulin secreted by a terminally differentiated plasma cell is the end result of a sequence of genetic decisions bearing both on its antigen specificity and its effector action.

The mechanisms underlying this sequence of decisions, and their relationship to other events in the life of a B lymphocyte were among the topics of discussion at a recent meeting* which covered most of the ground between the emergence of the first B lymphocyte in the fetus and the formation of an interacting network of immunoglobulin-secreting cells in the adult.

Commitment to the B cell lineage in avian embryos does not seem to occur until after the blood-borne stem cells have already migrated to the thymus (where T cells differentiate) and the bursa (where B cells differentiate)¹. The thymus can be shown to release a chemotactic factor which attracts stem cells and is shut off when a certain number have seeded the thymus. Bursal seeding begins only after the thymus is 'full'. But even at that stage, bursal cells are still capable of responding to thymic chemotactic factor: if unseeded thymic tissue is grafted into a chick embryo after bursal seeding has taken place, bursal cells will migrate to the new thymus and differentiate there (N.M. Le Douarin, Nogent-sur-Marne).

The nature of the signals that irrevocably commit a stem cell to a given lineage is not

known for either fetus or adult of any species; in mammals, B cell differentiation begins in placenta and fetal liver and continues in adult bone marrow where other haemopoietic cells also differentiate. Commitment to the B cell lineage is first detectable when immunoglobulin heavy chains appear in the 'pre-B' cells of the bone marrow or fetal liver². It is now known that the appearance of heavy chains signifies not only a decision to express immunoglobulin genes, but also a major decision on the antigen-specificity of the cell and its progeny. This follows from the discovery, first made in light chains^{3,4} but now extended to heavy chains⁵, that the expression of immunoglobulin genes can occur only after a chromosomal rearrangement which determines the primary sequence of the antigen-binding site.

Generation of antibody diversity

J.S. Seidman (National Institutes of Health) reviewed the evidence from mouse κ light chains that there are three modes of encoding antibody diversity. The complete coding region for the antigen binding site is constructed by the recombination of one of between 100 and 1,000 V genes at random with one of 5 J genes, with slight variations in the site of recombination. If similar recombination events take place independently between similar numbers of V and J genes on light and heavy chains, these sources of diversity could in theory account for the entire immunoglobulin repertoire, even without somatic diversification of hypervariable regions 1 and 2; especially in view of the fact that heavy chain expression precedes and is independent of that of light chains, so that a cell committed to a

*The INSERM Conference on B Cell Differentiation and Idiotypes took place at Seillac on September 30-October 4 1979.

membrane. Other receptors, such as those through which mitogens act, probably bind growth factors released by other lymphocytes. At what stages in ontogeny the cells respond to which stimuli is, however, still unclear, partly because of the problem of isolating cells at a defined stage. F. Melchers (Basel Institute for Immunology) was unable to shed any new light on early stages of B cell ontogeny but believes he has clarified the nature of the cell-cell signals that induce the T-cell-dependent production of antibody in B cells expressing surface immunoglobulin.

Three cell types and at least three signals are needed to induce antigen-specific, T-cell dependent immunoglobulin production by B cells. The three cells are the B cell itself, an antigen-specific T cell, and an antigen-presenting cell. The three signals are, first, the antigen; second, a surface antigen coded by the Ia region of the MHC complex and expressed on all three cells: this antigen must be recognised by the T cell as 'self' for the interactions to work (that is, they are MHC restricted); and third, a growth factor produced as a result of the interaction of antigen, T cell and antigen-presenting cell. Melchers has been able to show that the effect of the growth factor depends on the state of differentiation of the B cell. A small, 'resting' B cell that has not encountered antigen will respond with terminal differentiation to an antibody-secreting cell. After stimulation with antigen, the B cell becomes a larger, activated blast: blast cells respond to growth factor with both proliferation and antibody secretion.

One important implication of this schema is that the growth factor itself need not be antigen-specific: the antigen-specificity of the response resides in the induction of growth-factor production, and the induction of blast cell differentiation in B cells. In view of the controversy which has for some time surrounded the question of whether growth factors are, are not, or are sometimes antigen-specific, a system in which at least the cell types and their responses, if not the signals themselves, are defined, could provide important clarification.

On the other hand, this schema is only one among many and is unlikely to settle the issue definitively. Participants were quick to point out, for example, that at present this result can be obtained only with sheep red blood cells, and not with soluble antigens. The eventual clarification of such interactions may well depend on more and better definitions of subsets of cells.

Heavy chain switch

In the normal course of events *in vivo*, the activation of B cells by antigen probably also triggers the heavy chain switch. There is now mounting evidence that chromosomal rearrangements like that which brings about the V-J join also bring

about the heavy-chain switch. Honjo and Kataoka⁷ were the first to produce evidence, from the hybridisation kinetics of heavy chain mRNA probes with myeloma DNA, that the switch is accompanied by the deletion of the genes coding for the C regions expressed earlier in ontogeny. If deletion is the mechanism of the heavy chain switch, it follows, first, that the ordering of the C genes on the chromosomes should correspond to the order in which they are expressed in ontogeny; and second, that the switch should be irreversible. Genetic evidence from recombinant mouse strains however suggests that the δ chain gene is closer to the V genes than that of the μ chain (W.E. Paul, National Institutes of Health), which runs counter to the first prediction; and myeloma cells in culture seem to be able to switch back and forth between the three subclasses of γ chain (K. Rajewsky, Köln University), which runs counter to the second prediction.

However, the genetic evidence, while suggestive, is not conclusive, and the spontaneous reversion of the myeloma cells could be due to abnormal or unstable chromosome complements. In the meantime, evidence consistent with deletion continues to accrue from investigations on chromosomal DNA. Restriction analysis shows that J genes are near the μ gene in germline DNA⁸, and that μ genes have disappeared from myelomas secreting γ_1 chains, μ and γ_1 genes from myelomas secreting γ_{2b} chains, μ , γ_1 and γ_{2b} genes from myelomas secreting γ_{2a} chains, and μ and all three γ subclass genes from cells secreting α chains⁹.

Differences in membrane and secreted Igs

There is now good evidence that the last step in B cell differentiation, too, is marked by a change in the primary structure of the immunoglobulin molecule: membrane and secreted immunoglobulin are not the same. R.M.E. Parkhouse (National Institute for Medical Research, London) has used a charge-shift electrophoretic assay for detergent binding to show that the constant region fragments of membrane immunoglobulin M and immunoglobulin D have hydrophobic regions not present on the secreted molecule; and P. Vassalli (Geneva University) finds differences in the cyanogen bromide fragment patterns of membrane and secreted immunoglobulin M, though he declined to say in which part of the C region the difference lay. This is a matter of some importance because the membrane and secreted molecules seem to be identical at the C terminal¹⁰ and the difference must therefore be internal.

This question is not likely to be resolved by analysis of the chromosomal DNA, which has so far failed to reveal more than one gene for each immunoglobulin class. At present, it is widely suspected that the switch from membrane to secreted immunoglobulin is due to differential processing of the primary RNA transcript

in the nucleus. Since each of the four domains of the C region is coded by regions of DNA separated by noncoding intervening sequences, it is easy to see how differential splicing could produce changes within the C region and not only at its C terminal.

If the existence of one gene for each immunoglobulin class is confirmed, then strong support for the theory of differential splicing is provided by evidence for at least two¹¹ and perhaps as many as four¹² different immunoglobulin mRNAs in the cytoplasm; and B cells, which were among the first cells in which genes were discovered to contain intervening sequences, may prove to be invaluable in clarifying how the intervening sequences are removed in the course of gene expression. □

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100 years ago

AFGHAN ETHNOLOGY

THE events now in progress on the north-western frontier of British India have for the third time in this century directed the serious attention of statesmen, historians, and ethnologists to the remarkable people who give their name, or rather one of their names, to the north-eastern division of the Iranian table-land. During the empire of the Sassanides the whole of this region, was known as Khorasan, that is, Khoristan, the Land of the Sun or the East. This term, with the gradual reduction of the Persian sway, has shrunk to the proportions of a province on the north-eastern frontier of the Shah's estates, and has been replaced further east by the ethnical expressions Afghanistan and Balochistan, the lands of the Afghans and Baloches. . . the subjoined rough estimate of the population [of Afghanistan] according to nationalities will show that it is very far from being homogeneous:-

Afghans and Pathāns	Iranian stock	3,520,000
Tajiks	Iranian stock	1,000,000
Hindkis	Hindu stock	500,000
Hazaras and Aimaks	Mongolo-Tatar stock	600,000
Kataghāns	Türki stock	200,000
Badakshis	Galcha stock	100,000
Baloches	Iranian stock	100,000
Kizil-Bashes	Türki stock	75,000
Kohistan and Siah Posh	Galcha stock	50,000

6,145,000

From *Nature* **21**, 22 Jan., 276; 1880.

Exposure to lead in childhood: the persisting effects

from M.R. Moore

THE problem of assessing the long-term effects of environmental lead on the health of both adults and children is bedevilled by the difficulties of measuring exposure over long periods. The only likely contenders for useful measures of long-term lead exposure are concentrations in either bones or teeth and sampling of these presents considerable problems. The more easily measured blood lead concentrations or the various biochemical indicators of lead exposure provide an indication of current or recent levels of exposure only. But in determining the probable damage that will be caused, it is not only the length and severity of exposure that may be important but the age at which exposure occurs. Of all the persons in the community, the newborn child is the most prone to injury from overexposure to lead for several reasons, and the damage that may be caused then will have the greatest long-term social and economic consequences.

There are two distinct problems. First, that of overt lead poisoning, whose sometimes fatal effects are not in dispute. Second, and of greater interest at present, are the insidious effects of long-term lower level lead exposure to which various health effects have been ascribed, such as mental retardation, kidney damage and changes in cardiovascular function, but around which there is certainly some controversy about the levels of exposure at which these changes may begin to take place.

The scientific problem in these circumstances is to try and define some break-point in the continuum of exposure at which one can definitely say that injury has taken place. This is inevitably difficult since lead has always been and always will be present in the biosphere and will be taken into the body in food and drink. As there can be no question of reducing lead exposure levels to zero, scientific and medical policy must be the attainment of minimal risk of exposure.

One of the most important factors determining the eventual body-burden of lead is its rate of absorption from the gastrointestinal tract. Recent studies have clearly shown that this may be considerably influenced by the composition of the diet and is almost certainly greater than the commonly-quoted figure of 10% of the total ingested lead. A similar problem is also found in determining the absorption of inhaled lead. Although initial studies indicated pulmonary absorption leading to a rise in blood lead of $1 \mu\text{g}\%$ for every $\mu\text{g m}^{-3}$ of lead in the air, the most recent studies would suggest that the figure is at least twice this, but is totally dependent on

particle size and various other physical constraints, such as the volume of air breathed¹.

The problem of exposure in newborn and young children is particularly important as newly-born children, and indeed other mammals, absorb considerably greater percentages of lead from their diet than they do in adult life^{2,3}. And they can be exposed to lead in the environment through several routes. *In utero* the fetus is exposed to lead concentrations which are approximately the same as those found in maternal tissues, as the placenta is a poor barrier to lead⁴. After birth, the situation changes dramatically when two different populations of children can be seen. One is breast fed, the other is fed with prepared milk feeds, usually by reconstitution of dried milk powders with domestic water supplies. The concentration of lead in breast milk is approximately the same as that found in human plasma, that is, about one tenth of the concentration of lead in whole blood, and generally will constitute little hazard to children, even where maternal over-exposure has taken place. The lead concentration of all proprietary dried milk feeds is low. Where however these feeds are made up with water in which there are very variable, but often considerable concentrations of lead, the child may be exposed to very high concentrations of lead at a time of life when his or her developing tissues are most susceptible to any toxic insult. Where concentrations of lead in domestic water supplies are high, which occurs in many areas of the world through soft water supplies being delivered through lead plumbing (see ref. 5 for a recent study) the child is exposed to lead concentrations in the diet which on a weight per kg basis are greater than are likely to be experienced at any other time in his or her life and at a time furthermore, when at least twice as much of this dietary lead will be absorbed compared with later life.

The toxic effects of such exposure are not hard to find. Of all the developing tissues in the body, those most prone to such injury are the brain and the central nervous system which are those least able to repair themselves. A number of studies, both in animals and humans, now provide conclusive evidence that neurophysiological deficit will occur in children overexposed to lead. The exact degree of overexposure and change in body lead burden which may result in measurable changes remains to be defined.

The toxic effects of lead on the nervous system in these circumstances are probably biphasic. First, lead affects nervous tissue development and growth^{6,7}. These effects are almost certainly irreversible and will

result in permanent neurological change, especially where the exposure has taken place early in life. Second, lead acting on various biochemical systems will alter concentrations of neuro-active metabolites. This type of change is probably reversible. Proof already exists that such changes can take place within the haem-biosynthetic pathway with overproduction of δ -aminolaevulinic acid, now shown to be neuro-active⁸, and in the adrenergic and cholinergic biosynthetic systems with consequent alterations in neurotransmitter concentrations.^{8,9}

In the earliest studies of childhood overexposure to lead it was clear that classroom performance was poorer than in children that had not been exposed. Thereafter, studies showed that mental retardation could be linked with overexposure to lead in drinking water and with high concentrations of blood lead at about the time of birth. Studies in El Paso, Texas linked blood lead concentrations of between 40 and 68 μg per 100 ml with diminished classroom performance (adjusted for age) although there was some controversy over the interpretation of these results.

In the most recent studies a similar degree of diminished learning ability (IQ drop of 4-7 points) and behavioural changes such as diminished attention span, have been associated with increased concentrations of lead in teeth. These studies by Needleman *et al.*¹⁰ and Winnecke¹¹ are the most convincing evidence to date of low level effects, since the use of tooth lead reflects long-term rather than recent exposure in these children. Children with greater exposure to lead performed significantly less well in the Wechsler Intelligence Scale for Children Test. Although tests for gross motor coordination were not done in Winnecke's study in Germany, changes in classroom behaviour were observed in Boston by Needleman *et al.* The importance of these studies lies in the battery of tests applied and in the matching and allowance for non-lead related variables such as weight, height and socioeconomic class, which were controlled in the analysis of covariance.

The emphasis now being placed on the study of the effective dose-response relationship between lead exposure and possible neuropathological effects in children reflects the fact that the overexposure to lead of young children will have the greatest potential economic

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effects through increased requirement for care in later life in those most severely affected and by diminution of intellectual potential. Children are inevitably the unwitting victims of adults' use of lead, especially in industrialised communities and in areas of heavy traffic.

It is impossible to put any value whatsoever on human potential and development but it is safe to say that for any community, the economic burden of looking after persons with mental deficit is high and is increasing. This burden should certainly not be unnecessarily loaded with persons in whom such deficit is avoidable.

In industry, the hazards of over exposure to lead are easily perceived and a solution simple, even if expensive to achieve. But this does not apply to environmental exposure. The simplest cause of environmental exposure to remove would be lead

in automobile fuel. This course of action would certainly carry a high potential economic toll and lead in petrol is only one limited source of exposure in Western society. To remove other sources such as lead plumbing would be even more expensive, which must inevitably be balanced against the less easily quantified cost of doing nothing. Perhaps the ultimate aim of any economic as opposed to medical policy in this sphere must be to balance the cost of a healthy community against the costs of achieving a healthy environment. It is particularly poignant as the International Year of the Child comes to an end that children who have so little economic or political muscle, but in whom the toxic effects of over exposure to lead are most manifest should thus have the greatest potential long-term economic effect on the community. □

Body temperature, activity and energy costs

from R.D. Martin

LIVING birds and mammals are distinguished as a group by their maintainance of a high body temperature with the generation of heat in association with a high metabolic turnover. Until recently, however, attempts to provide a convincing hypothesis to account for the evolution of this phenomenon of endothermy have remained inconclusive. Now, information from a number of disparate fields of research seems at last to point the way towards a synthetic theory accounting for the emergence of endothermy despite its high energetic cost.

A major stimulus for re-examination of possible reasons for the evolution of endothermy in birds and mammals comes, strangely enough, from a recent suggestion that this feature had also been developed among the dinosaurs (Bakker *Evolution* 25, 636; 1971; *Nature* 238, 81; 1972). Bakker's arguments supporting endothermy in dinosaurs (invoking raised body posture, inferred predator/prey ratios indicating high energy requirements, and bone histology indicative of rapid growth) have encountered criticism from several quarters, but regardless of its specific merits the hypothesis had the advantage that it focused attention more on the topics of locomotor activity, energy requirements and overall metabolic turnover. As Hopson (*Paleobiology* 2, 271; 1976; *Rev. ecol. Syst.* 8, 429; 1977) concluded, it seems likely that the dinosaurs were not comparable with birds and mammals in terms of body temperature regulation, but they were undoubtedly peculiar in some aspects of their metabolic house-keeping in

comparison with modern reptiles. Thus, any synthetic explanation of the origins of endothermy should ideally account not only for the relevant features of birds and mammals, but also for any identifiable peculiarities of the dinosaurs concerning overall energy relationships.

In diametric opposition to the synthetic trend stimulated by Bakker's work, a special explanation for the evolution of endothermy in mammals alone was subsequently proposed by A.W. Crompton, C.R. Taylor and J.A. Jagger (*Nature* 272, 333; 1978). Their model involved a two-step acquisition of endothermy: (1) invasion of a nocturnal niche, with development of a moderately elevated, controlled body temperature (25–30 °C), but without any significant increase in resting metabolic rate; (2) re-invasion of a diurnal niche, with further elevation of the controlled body temperature (to close to 40 °C) and an obligatory increase in resting metabolic rate.

One important piece of evidence cited in support of this hypothesis consisted of new experimental evidence that three extant placental insectivores (*Tenrec ecaudatus*; *Setifer setosus*; *Erinaceus europaeus*) exhibit a 'reptilian-type' pattern of oxygen consumption during locomotion. This feature was interpreted as a primitive retention in these nocturnal insectivores illustrating the first step in the acquisition of endothermy. However, a nocturnal monotreme (*Tachyglossus aculeatus*) and a nocturnal marsupial (*Didelphis virginiana*) studied under the same conditions exhibited a typical 'mammalian-type' pattern of oxygen consumption during locomotion. Thus the two-step model can only account for the conditions in these two species (and

for the vast majority of nocturnal placental mammals which presumably have 'mammalian-type' energetics) by postulating numerous intermediate diurnal ancestors in which the second step would have been accomplished. An alternative explanation is that the tenrecs and the hedgehog exhibit 'reptilian-type' energetics during locomotion for special reasons unrelated to the overall framework of evolution of endothermy in mammals. In any event, the two-step model cannot be applied to the parallel evolution of endothermy in birds (for which no ancestral nocturnal phase can be envisaged) and Crompton *et al.* themselves point out that their hypothesis explicitly rules out any possibility of early trends towards endothermy among therapsids (mammal-like reptiles).

The need for an *an hoc* explanation for the separate evolution of endothermy in mammals has now been questioned in a paper by A.F. Bennett and J.A. Ruben (*Science* 206, 649; 1979), which concentrates on the high energetic cost of endothermy and the relationship between high metabolic rate and increased capacity for aerobic respiration. The high energetic cost of endothermy in birds and mammals demands that it have a substantial selective advantage. Bennett and Ruben propose that this advantage lies in the far greater stamina in locomotion permitted in vertebrates with high metabolic rates and an associated greater capacity for aerobic respiration.

Taking the same linear relationship between oxygen consumption and speed of locomotion exploited by Crompton *et al.* in their experimental work, Bennett and Ruben have shown that a plateau of oxygen consumption is reached at a critical speed of locomotion. For any given body weight, a reptile will attain this plateau at a much lower speed than a mammal and thus be constrained to carry out anaerobic respiration at an earlier stage. (Interestingly, the relationships between oxygen consumption and running speed determined for the placental insectivores studied by Crompton *et al.* reveal no attainment of an anaerobic plateau, so these mammals are apparently not of the 'reptilian-type' in this respect). Hence acquisition of greater locomotor stamina may well provide a unitary explanation for the evolution of endothermy in the face of greatly increased energy consumption.

Two important corollaries are identified by Bennett and Ruben. First, since locomotor stamina is undoubtedly a prerequisite of bird flight, it would follow that endothermy probably evolved among the ancestors of birds before the emergence of flight. Second, since increased metabolic turnover is directly related to a decrease in the proportion of resources which can be allocated to reproduction by any individual, it is likely that endotherms would be particularly subject to selection pressure favouring the emergence of

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secondary mechanisms to enhance infant survival. The characteristic presence of parental behaviour in birds and mammals (associated with viviparity in the latter) may thus be linked to the presence of endothermy.

Going beyond the material presented by Bennett and Ruben, it is also noteworthy that there is probably a close connection between metabolic turnover, activity levels, aerobic capacity and brain size in birds and mammals. The fact that birds and mammals have relatively larger brains than reptiles, amphibians and fish (Jerison *Evolution of the Brain and Intelligence*, 1973) could relate directly to greater locomotor capacity and to the high metabolic levels associated with endothermy. If a single such explanation covering all these aspects is applicable to living birds and mammals, these corollaries (and others as yet unidentified) could considerably increase our understanding of vertebrate evolution. The present controversy over the presence or absence of endothermy in dinosaurs, having stimulated the search for new explanatory principles, may itself find an acceptable solution within this unitary framework, bringing in such topics as relative brain size (Hopson *op.cit.*, 1977; Jerison *op.cit.*, 1973) and evidence for parental behaviour in dinosaurs (Hopson *op.cit.* 1973; Horner & Makela *Nature* 282, 296; 1979). □

Switchboards and folds

from P.D. Calvert

SEMICRYSTALLINE polymers derive their useful properties of strength and toughness from their structure of alternating crystalline and rubbery layers. Hence the way in which the chains are arranged in these layers and bond them together is of importance in guiding our thinking about many aspects of their behaviour.

Small angle neutron scattering (SANS) results on crystalline polyethylene published in 1976 showed that current ideas on the arrangement of the molecular chains in crystalline polymers needed rethinking (see *News and Views* 263, 371; 1976). Polymer crystals are usually thin, about 10 nm, in the direction traversed by the chains so it is agreed that chains must fold back for several traverses through any crystal. Before 1976 it was widely believed that the chains were folded tightly to give adjacent re-entry with little loose material at the crystal surface. The alternative was the 'telephone switchboard' model where the chains re-entered the crystal at some distance from their exit point. The Faraday Discussion on 'Organisation of Macromolecules in the Condensed Phase' held in Cambridge last autumn gave a clear picture

of developments over the past 3 years.

The basic observation by neutron scattering, that there is little change in the radius of gyration of polyethylene on crystallisation, was originally reported by Ballard and coworkers at ICI. They have since shown that polypropylene behaves similarly and workers at Bristol and at Mainz have confirmed the polyethylene result. At Cambridge, J. Guenet, C. Picot and H. Benoit from Strasbourg reported similar results for deuterated polystyrene chains in a high molecular weight ($M_w = 1.7 \times 10^6$) polystyrene matrix. However, in a matrix of lower molecular weight ($M_w = 4 \times 10^5$) the radius of gyration increased by up to 40% on crystallisation. This is of great interest because the fact that no change in radius of gyration is usually observed means that there is little with which really to test theories. One problem with SANS is that segregation of the labelled molecules may occur on crystallisation, but the Strasbourg group claims that this is not so in their samples.

Several different approaches have been successful in fitting the SANS data. D. Yoon and P. Flory (Stanford University) have shown that switchboard models with or without some low probability of adjacent re-entry can fit the data. The Mainz group has used a 'solidification' model in which straightening of chain sections is superimposed on the initial, molten random coil. The Strasbourg group has fitted its data by allowing adjacent re-entry over the central sections of the chain and leaving amorphous 'wings' at either end. The US National Bureau of Standards group here had success with this and with a cluster model, where clusters of three or four adjacent folds are linked by longer loose folds. The lesson seems to be that the many different models can be fitted to the data available so far, which can only be used to eliminate some possibilities. However, matters should improve as measurements are extended up to 'intermediate' angles where the scattering vector is commensurate with the individual folds and clusters rather than the whole chain. Careful measurements of absolute, rather than relative, intensity may also be necessary in these regions.

The ICI/Jülich group has looked carefully at the behaviour of the radius of gyration at low molecular weights in polypropylene (*Polymer* 20, 399; 1979). In principle, the chain might be expected to resemble a straight rod when it becomes so short that it can only fold once or twice. Then the rod length should be equal to the lamellar spacing seen by small angle X-ray scattering. In fact it is about twice this. This they have explained in terms of chains running through pairs of lamellae. However, we are rather ignorant about the way in which the stacks of lamellar crystals form; it often seems to be a two-step process with later filling-in by small lamellae of the spaces between those which form first. Thus it is difficult to see how

really to view this interesting result. This group also reported data on drawn polypropylene where a six-fold draw ratio resulted in a decrease of 3–4 times in the radius of gyration perpendicular to the draw direction, more than the decrease of $\sqrt{6}$ that might be expected. There was also a decrease of 3–6 times in the molecular weight measured by SANS although the chains were unbroken suggesting that the chain was now acting as a series of separate beads.

Given that interpretation of the SANS data on melt-grown crystals is very dependent on the models used, one would hope for signs of theoretical advances. Here the meeting at Cambridge had little to offer. The interpretation of the results of other experimental observations in terms of the switchboard and adjacent re-entry models were debated, as they have been for many years, but little progress has been made. A great deal of information exists but most of it can be interpreted in terms of more than one model. However, it still remains unclear whether the telephone switchboard model can be reconciled with sensible packing densities close to the crystal surface. Recently Flory suggested that there was not time for a full adjacent re-entry conformation to be adopted during crystallisation, given the relaxation time of a whole chain. J. Klein and E. Di Marzio argued against this, the former by calculating the diffusion rate of a chain in a tube and the latter by calculating the time to pull a chain through a hole in a plane, but as all the calculations are on different bases it is difficult to grasp the reasons for the contradictions. A telling point is that polymeric impurities can be pushed aside even during rapid crystallisation. It is worth giving more thought to the relationship between the time required for this process and that for crystallisation.

Studies on solution-grown polyethylene single crystals seemed to have reached a reasonable level of agreement. Yoon, D. Sadler (Bristol) and M. Stamm (Mainz) all talked in terms of chains re-entering close to their point of exit and spread over a few neighbouring crystal planes, in other words not 'adjacent' re-entry but 'nearby' re-entry. However, there are still differences in the form of the models used by the different groups.

The SANS results have changed most people's picture of the crystal surface. They now picture it with many loose loops whereas previously the majority viewpoint was that the surface was virtually smooth. For the future we must await more detail from the neutron scattering at intermediate angles. The most viable theoretical approach seems to me to be to start from the molten random coil and to interpret the action of the kinetics and thermodynamics of crystallisation and annealing but progress has been slow so far. □

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REVIEW ARTICLE

Thermal X-ray emission from neutron stars

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The Einstein Observatory (HEAO 2) presents the first realistic opportunity to see a neutron star by observing radiation from its surface. In this review we show that the detection of such thermal radiation or the establishment of strong upper limits to neutron star temperatures is of considerable importance to the study of neutron star matter, neutron star structure, and polar magnetospheres.

WHEN the existence of neutron stars was first suggested¹⁻³, it seemed likely that these objects, with a radius of only 10 km, would forever remain in the realm of theoretical speculation. Observational advances in the past decade have rendered this view obsolete. Neutron stars have been either established or suggested as the underlying object for many astrophysical phenomena: pulsars; X-ray binaries, transients, and bursters; γ -ray bursters; and even active galactic nuclei. Nevertheless, with the possible exception of the decaying tails of X-ray bursts⁴, we have yet to 'see' a neutron star in the sense of detecting its surface radiation⁵. Such an observation may well now be possible.

We review here the status of theories concerning the thermal properties of neutron stars with a view towards establishing how observational astronomy may contribute to future studies. Table 1 presents the photon fluxes expected in several regions of the spectrum from a 10 km neutron star at a distance of 100 pc (equal to that of the closest radio pulsar). The legend to Table 1 lists the limiting sensitivities of the best available instruments in each spectral regime. While the Space Telescope will substantially lower sensitivity thresholds in the visible and near UV, much work can already be done in the soft X-ray region with the imaging telescope aboard the Einstein Observatory (HEAO 2).

There are several reasons why neutron star surfaces are thought to be hot enough to emit soft X rays ($T \sim 10^6$ K). At its formation in the imploding core of a supernova, a neutron star will be at a temperature $> 10^{11}$ K. It cools rapidly at first through various neutrino processes, but most recent calculations⁶ suggest that the surface temperature remains above one million degrees for at least 10^3 yr and above 10^5 K for perhaps 10^5 yr. If this is the case, several neutron stars should be detectable in young and/or nearby supernova remnants. Furthermore, several heating mechanisms have been proposed which may maintain surface temperatures of several hundred thousand degrees or higher in older neutron stars (such as radio pulsars). These include heating of the polar caps via bombardment of the surface with relativistic particles from the magnetosphere, frictional dissipation from the crust-superfluid interface, microquakes and accretion from the interstellar medium.

Heating sources

Initial cooling: Although neutron stars are thought to form at very high temperatures ($T > 10^{11}$ K) in the core of a supernova explosion, much of this initial thermal energy is radiated away in neutrinos during the first hours, leaving a 1-day-old star with an internal temperature of 10^9 – 10^{10} K. The thermal history of neutron stars has recently been reviewed by Tsuruta⁶. Cooling rates were calculated for various neutron star equations of state, stellar masses and magnetic field strengths. Both photon and neutrino cooling were considered; neutrino processes included were neutrino bremsstrahlung, modified URCA and plasmon processes, as well as minor processes such as photo production of neutrinos, pair annihilation and neutrino synchrotron radiation. The effects of the star's magnetic field and the superfluid nature of its interior were taken into account. In addition to a range of standard equations of state, stars containing pion

condensate cores were considered. The thermal properties of more exotic equations of state, including those for pure quark stars, have been studied by Brecher and Burrows⁷.

The result of such cooling calculations is a curve giving the neutron star surface temperature as a function of time. Neutrino cooling dominates for at least the first 10^3 yr in all models, so any effect of the magnetic field on photon opacity is of minor consequence. The range of temperatures allowed by standard nuclear models after 350 yr is from 3.5 to 15×10^6 K; after 10^4 yr, the temperatures fall to between 2 and 5×10^6 K. As the result of a less sensitive temperature dependence (T^6 instead of T^8 as for other major processes), neutrino cooling due to pion decays allows the star to reach lower temperatures more rapidly. Although the density ρ_π at which neutron star matter makes a transition to a pion condensate is very uncertain (3×10^{14} g cm⁻³ $\leq \rho_\pi \leq 3 \times 10^{15}$ g cm⁻³ with $\rho_\pi \sim 6 \times 10^{14}$ g cm⁻³ as the best current estimate⁸), one can compute the maximum cooling effect of pions by choosing the lower limit of 3×10^{14} g cm⁻³, thus allowing the largest fraction of the stellar mass to be in this state. These maximum pion cooling models yield temperatures of 8×10^5 K and 3×10^5 K after 300 yr and 10^4 yr, respectively⁶.

An analytical estimate of the cooling equations by Brown⁹ yielded a surface temperature for the Crab pulsar of $\sim 4 \times 10^6$ K, consistent with the results of Tsuruta's⁶ full numerical integrations and only slightly above the measured upper limit of 3.0×10^6 K (ref. 10). Malone¹¹ and Maxwell^{12,13} have also studied the cooling problem, and the results are all qualitatively similar. They differ by less than a factor of 2 from the results described above—the discrepancies apparently arise from small differences in the input physics: for example, the choice of an equation of state (Maxwell¹² assumes a uniform density star). However, many of these calculations use core-crust thermal conductivities calculated over seven years ago¹⁴. Recent work by Ray¹⁵ finds significantly lower conductivity values and thus predicts lower temperatures for recently formed stars. His most extreme model predicts surface temperatures of 1.7×10^6 K and 1.3×10^6 K for stars of age 300 yr and 1,000 yr, respectively. Also, since the core-to-surface thermal conduction time scale is $\sim 10^3$ yr, one cannot distinguish, in these models, between young stars with and without pion condensate cores.

Brecher and Burrows⁷ have recently made the first detailed calculations for the cooling of quark stars. These stars tend to have a higher heat capacity, but also tend to cool slightly faster, resulting in predicted temperatures quite similar to those discussed above; a 10^3 -yr-old star has a temperature of $\sim 5 \times 10^6$ K. As a result of their higher heat capacity, the stars also tend to remain warm longer. For instance, after 2 Myr (a typical age for radio pulsars), the surface is still at 10^6 K. However, it is uncertain whether a quark star contains a large superfluid component, and, as for normal matter stars, the inclusion of superfluidity effects in these calculations would probably lower these predicted temperatures. The thermal properties of the recently proposed 'abnormal' matter neutron stars¹⁶ have not yet been calculated.

Table 1 Thermal neutron star photon fluxes in the visible, far UV, and X-ray bands*

T (10^6 K)	Flux		
	Visible band†,‡ (3,000–10,000 Å) (mag)	Far UV band‡ (1,000–2,000 Å) (mag)	X-ray band§ (0.2–3.0 keV) (photons $\text{cm}^{-2} \text{s}^{-1}$)
0.1	28.5	25.8	1.3×10^{-11}
0.3	27.3	24.1	1.9×10^{-4}
1.0	26.0	22.7	0.23
3.0	24.8	21.4	12

* A neutron star radius of 10 km and a distance of 100 pc are assumed; interstellar absorption has been neglected.

† Skylight limits the sensitivity of ground-based telescopes to $m_v \sim 23$.

‡ The wide field camera on the Space Telescope is expected to be able to detect 27th mag stars in the far UV and visible regions of the spectrum.

§ The imaging proportional counter on the Einstein Observatory has a sensitivity of 2.5×10^{-5} photons $\text{cm}^{-2} \text{s}^{-1}$.

Virtually all of the standard calculations of neutron star cooling rates, therefore, suggest surface temperatures of several million degrees for a 300-yr old object, with temperatures remaining within the range $5\text{--}20 \times 10^5$ K for at least 10^4 yr. The only previous observation applicable to these theories has been the upper limit of $\sim 3 \times 10^6$ K for the Crab pulsar from the lunar occultation measurements^{10,17}. With the imaging capabilities and thousandfold increase in sensitivity offered by the Einstein Observatory, we can now expand this data base significantly. A search of nearby supernova remnants will either detect point sources and yield surface temperatures to be used as constraints on cooling theories, or provide upper limits nearly an order of magnitude lower in temperature than previously possible and delimit the viable choices for neutron star equations of state (for example, by distinguishing between the presence or absence of pion condensates).

Figure 1 presents the minimum surface temperatures required for neutron stars to be detected in 10^4 -s pointings with the Einstein Observatory imaging proportional counter (IPC) (see ref. 18 for a detailed description). These temperatures were determined by requiring that the number N of photons in a 2 arc min radius detection cell be at least 25 [a 3σ result for the typical measured IPC background rate of 0.005 counts (s cell)⁻¹]. For a thermal isotropically radiating neutron star at a distance d with temperature T_0 and radius R_0 , N is given by

$$N(T_0) = \frac{2\pi(1+z)^2 R_0^2 t}{c^2 d^2 h^3} \int_{E_\infty^{(1)}}^{E_\infty^{(2)}} E_\infty^2 A(E_\infty) \tau(n, E_\infty) dE_\infty \quad (1)$$

$$\times \exp[(1+z)E_\infty/kT_0] - 1$$

where t is the observing time. The integral is over photon energies E_∞ within the bandwidth, $E_\infty^{(1)}$ to $E_\infty^{(2)}$, of the detector. The subscript ∞ emphasises that the photon energies are as measured by an observer at infinity. On the other hand, T_0 and R_0 (the 'true' temperature and radius) are defined, in the usual convention, at the surface of the neutron star. We have used the relationships

$$\begin{aligned} T_\infty &= (1+z)^{-1} T_0 \\ R_\infty &= (1+z) R_0 \end{aligned} \quad (2)$$

where T_∞ is the temperature measured by the distant observer and R_∞ is the apparent radius that he would deduce from the black body law (see ref. 19). The factor $(1+z)$ is due to the gravitational redshift and, at the surface of the star, is given by

$$1+z = \left(1 - \frac{2GM}{c^2 R_0}\right)^{-1/2} \quad (3)$$

where M is the mass of the star (see ref. 20). Finally, $A(E_\infty)$ is the effective area of the detector and $\tau(n, E_\infty)$ is the transmission of the interstellar medium as a function of the number density n of neutral hydrogen atoms along the line of sight. We have

plotted results for $n = 0.3$, $n = 1$, and $n = 3$ atoms cm^{-3} . The absorption coefficients used were those of Brown and Gould²¹.

For all of the present calculations, we have adopted a neutron star mass of $M = 1.3M_\odot$ and a radius $R_0 = 16$ km. This value for M is used in much of the theoretical work described above and is also consistent with the measured masses of neutron stars found in binary systems^{22,23}. The adopted radius is that appropriate to this stellar mass, using either the mean field²⁴ or tensor interaction²⁵ equations of state²⁶. The value of the redshift for this case is $z = 0.13$.

Our assumption of isotropic black body emission for the thermal radiation from a hot neutron star is clearly an oversimplification. The strong magnetic field produces a nonuniform thermal conductivity over the surface of the star leading to significant temperature gradients. Brinkmann²⁷ has also recently described the deviation from a black body spectrum which results from the effect of the field on the optical properties of the surface. He finds that the effects are not large for stars with fields $\geq 10^{12}$ G, but the strong dependence of the surface index of refraction on the values of the cyclotron and plasma frequencies causes the expected radiation to be lowered by more than a factor of 5 from the black body value for $B \sim 5 \times 10^{11}$ G. A measurement of the actual emitted spectrum would reveal much about the properties of neutron star surface material and the strength of the magnetic field.

Polar cap heating: The origin of pulsar radio emission has been one of the most difficult theoretical problems in astrophysics of the past decade. However, models have emerged which agree on several fundamental points. Based on the original work by Sturrock²⁸, these models invoke pair creation by the γ rays produced via the electrostatic acceleration of primary particles from the magnetic polar caps of the star. Although the location of the region of pair production and many other details of the models differ^{28–35}, all of the models require that a large back-flux of γ rays and relativistic particles be incident on the pulsar's polar cap surface. This flux will heat the polar cap to soft X-ray temperatures (0.1–1 keV) and, assuming that a large fraction of this energy is not lost by heat conduction into the stellar interior, may render the polar cap region visible at distances of up to ~ 1 kpc. Owing to uncertainties in the distance from the stellar surface at which pulsar radiation in various spectral regimes arises and the values of some parameters in the polar cap theories, we ignore general relativistic corrections in this section; that is, we drop the distinction between R_0 and R_∞ , T_0 and T_∞ , and so on.

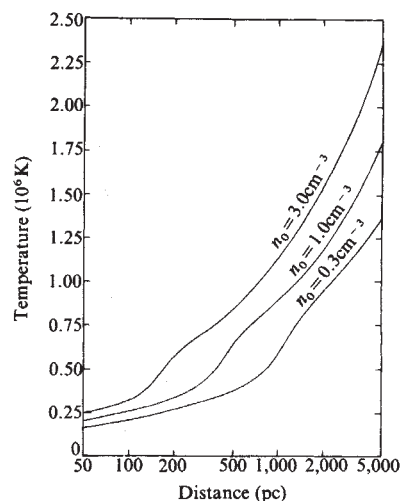


Fig. 1 Temperatures for a 16-km neutron star that produce detectable soft X-ray fluxes (25 counts) in a 10^4 -s pointing of the Einstein Observatory IPC. Curves for several representative values of the interstellar neutral hydrogen density, n_0 , have been plotted for T as a function of distance. Note that there are 50 known radio pulsars within 500 pc of the Earth.

Table 2 Predicted X-ray temperatures and fluxes for potential γ -ray emitting pulsars

Name PSR	B_{12}/P^2	D (pc)	$\dot{E}$ (10^{33} erg s $^{-1}$)	A (10^{10} cm 2)	T (10^6 K)	IPC counts in 2,000 s (0.1–5 keV)
0355+54	35	1,500	46	1.7	7.0	900
0531+21	3,400	2,000	4.6×10^5	8.2	7.6*	2,900*
0540+23	33	2,800	41	1.1	7.6	180
0611+22	40	3,500	64	0.82	9.1	140
0740–28	61	2,000	150	1.6	7.6*	570*
0833–45	430	500	7,000	3.0	6.7*	16,000*
1055–52	31	1,100	37	1.4	7.0	1,500
1449–64	50	2,600	97	1.5	8.7	450
1557–50	27	9,000	28	1.4	6.5	5.8
1641–45	15	4,900	8.5	0.59	6.0	9.6
1727–47	17	5,900	11	0.32	7.4	7.2
1742–30	15	2,900	8.6	0.73	5.7	38
1826–17	14	9,400	7.7	0.87	5.3	1.3
1831–03	11	9,400	5.1	0.39	5.8	0.87
1844–04	16	4,900	9.7	0.45	6.6	11
1907+10	11	5,000	4.6	0.96	4.5	4.1
1913+10	15	8,400	9.2	0.67	5.9	2.3
1914+09	11	2,000	5.1	1.0	4.6	53
1914+13	13	7,900	6.5	0.96	4.9	1.6
1915+13	32	3,200	39	1.4	7.0	120
1916+14	11	1,000	5.1	0.22	6.7	250
1929+10	10	110	4	1.2	4.2	29,000
1930+20	15	6,900	8.7	1.0	5.3	3.7
1930+22	140	8,100	770	1.9	14	130
1933+16	12	6,000	5.2	0.75	5.0	3.1

* Values calculated using measured values of L_γ (see text). In other cases, $L_\gamma = \frac{1}{2}\dot{E}$ was assumed.

The models of Ruderman and coworkers^{29–32} offer a choice of two regions of the magnetosphere from which polar cap-heating particles emerge. In the so-called ‘outer gap’ picture^{31,32}, a large flux of positrons (electrons) originates in a region of magnetospheric charge reversal at a radius of $\sim 0.2 R_c$ (where R_c is the light cylinder radius) and impinges on the polar cap, heating it to temperatures of $\sim 10^7$ K. This model accounts well for the high energy γ radiation from the Crab and Vela pulsars as well as for the 100 MeV γ -ray pulses recently reported from two older pulsars (PSR0740–28 and PSR1822–09)³⁶. The condition for such outer-gap induced γ -ray emission is

$$B_{12}/P^2 > 10 \quad (4)$$

where B_{12} is the pulsar’s dipole magnetic field strength in 10^{12} G, and P is the period in seconds³². Of the 110 pulsars in the Galaxy with measured period derivatives (necessary to estimate B_{12}), the 25 sources which satisfy this criterion are listed in Table 2, along with their values of B_{12}/P^2 , distance, and spin-down energy loss rates. From the four observed γ -ray pulsars, it seems that the efficiency with which the rotational kinetic energy lost by the star is converted to γ rays, $\eta = L_\gamma/\dot{E}$, is a strong function of B_{12}/P^2 : $\eta = 1/3,000$ for the Crab ($B_{12}/P^2 = 3,500$), $\eta = 1/200$ for Vela ($B_{12}/P^2 = 425$), $\eta = 0.2$ for PSR0740–28 ($B_{12}/P^2 = 60$), and $\eta = 1.0$ for PSR1822–09 ($B_{12}/P^2 = 11$). For nearly all of the pulsars listed in Table 2 which have not been detected as γ -ray emitters, B_{12}/P^2 is in the range 10–50. By adopting a value of $\eta = 0.5$ for these sources, we can calculate the expected γ -ray flux at the Earth for each object: in every case, the expected flux is below the threshold detection level for COS B of $\sim 5 \times 10^{-11}$ erg cm $^{-2}$ s $^{-1}$, so the supposition that all of these sources may display outer-gap discharges is not in conflict with present γ -ray observations.

M. A. Ruderman (personal communication) estimates that the thermal X-ray heating L_x of the polar cap resulting from the bombardment of charged particles will be $\sim 0.1 L_\gamma$. This assumption, together with L_γ and the polar cap area, defines the temperature of the polar cap. For the area A of a single polar cap shown in column 5 of Table 2, we have used³⁷

$$A = \pi R^2 (\Omega R/c) \quad (5)$$

where Ω is the pulsar angular spin frequency, c is the velocity of light, and we take $R = 16$ km as before. Column 6 shows the derived temperature of the polar cap [see equation (7)], where we have used the measured value of L_γ for the four γ -ray pulsars and have taken $L_\gamma = \frac{1}{2}\dot{E}$ otherwise.

Note that, with a single possible exception, the X-ray fluxes implied by the temperatures and areas in Table 2 are all well below the sensitivities of previous X-ray sky surveys (The sensitivities of such surveys have typically been of order 1 Uhuru flux unit (UFU) = 1.7×10^{-11} erg cm $^{-2}$ s $^{-1}$ in the 2–10 keV band.) The exception is 1929+10, for which the predicted 2–10 keV flux should have been only marginally detectable and for which the value of B_{12}/P^2 is close to the cutoff. There is therefore clearly no serious conflict between the predictions of the model and the existing data.

The final column of Table 2 shows the number of 0.1–5.0 keV X-ray counts in the Einstein Observatory IPC expected in a 2,000-s observation. The calculations were performed as described above assuming an interstellar neutral hydrogen density of 1 cm $^{-3}$ and with A as in column 5. We have taken into account the change in the projected surface area of the polar cap as the star rotates and have averaged radiation from both poles of the star by integrating the contribution from a random distribution of angles between the magnetic pole and the rotation axis. Clearly at least half of the sources would be easily detectable in such short observations. Even if the conversion efficiency is as low as $\eta \sim 0.01$, several objects could still be detected in 10^4 -s pointings. Such observations will thus apply a stringent test to this or any similar models for γ -ray production in radio pulsars.

Pulsars with values of B_{12}/P^2 below the cutoff of ~ 10 still produce X-ray emission. In the inner-gap models of Ruderman and Sutherland²⁹, the predicted X-ray luminosity is given by

$$L_x \approx 1 \times 10^{30} \frac{\epsilon \cdot B_{12}}{P^2} \text{ erg s}^{-1} \quad (6)$$

where ϵ is related to the binding energies of the atoms in the crust of the polar cap ($10^{-3} < \epsilon < 1$). Thus, expected X-ray luminosities range from $\sim 10^{32} \epsilon$ erg s $^{-1}$ for a 0.25-s pulsar with $B \sim 5 \times 10^{12}$ G down to $5 \times 10^{29} \epsilon$ erg s $^{-1}$ for a 1-s source with a field of 5×10^{11} G.

Assuming a black body spectrum for the radiation, we expect a temperature

$$T = \left(\frac{L_x}{A\sigma} \right)^{1/4} = 0.65 \times 10^6 \left(\frac{L_x}{10^{28} \text{ erg s}^{-1}} \right)^{1/4} \left(\frac{A}{10^9 \text{ cm}^2} \right)^{-1/4} \text{ K} \quad (7)$$

where σ is the Stefan–Boltzmann constant [5.67×10^{-5} erg cm $^{-2}$ s $^{-1}$ K $^{-4}$] and A is the surface area of the polar cap. For even the closest pulsars, previous X-ray results are no help in setting a limit on ϵ : for PSR0950+08 with $P = 0.25$ s, $B = 5 \times 10^{12}$, and $D = 110$ pc, the flux is < 1 UFU for $\epsilon < 0.5$, and for all other pulsars, $\epsilon = 1$ is allowed. Theoretical arguments (M. A. Ruderman, personal communication) suggest values for ϵ of $\sim 1/500$ for the fastest pulsars ($P < 0.1$ s) but allow ϵ to approach unity as the star ages.

The model of Arons and Scharlemann³³ predicts the lowest X-ray flux of any of the polar cap models suggested thus far. The total X-ray luminosity L_x is given by

$$L_x \approx 2 \times 10^{30} \left(\frac{0.1}{P} \right)^{27/8} \text{ erg s}^{-1} \quad (8)$$

Thus, for this model, predicted luminosities range from 3×10^{30} erg s $^{-1}$ for the Vela pulsar to 10^{28} erg s $^{-1}$ and 8×10^{26} erg s $^{-1}$ for stars with 0.5-s and 1.0-s periods, respectively. The implied temperatures fall between 0.4 and 2×10^6 K. As recently noted by Arons³⁵, equation (8) provides a lower limit to polar cap heating in the context of pair production models. The heating could be greater due to trapping of positrons in a region above the pair creation zone³³ or because of unsteady spark discharges as in the Ruderman and Sutherland models.

Table 3 lists the expected Einstein Observatory IPC counts in a 10^4 -s observation for a range of polar cap temperatures. A polar cap area of 10^{10} cm² and an interstellar neutral hydrogen density of 1 cm⁻³ have been assumed. Setting our detection threshold at 25 counts, we see that only relatively nearby (≤ 1 kpc) sources can be observed, primarily because these low energy X rays (10^6 K ~ 0.1 keV) are absorbed by the interstellar medium. From a compilation by J. H. Taylor (personal communication) of 325 known pulsars, we can expect only marginal detections under the assumptions of the Arons and Scharlemann model³³. For the Ruderman and Sutherland theory, on the other hand, we can expect up to 20 strong detections, depending on the value of the parameter ε .

Dynamical friction: The widely quoted 'two-component' model of neutron star structure³⁸ suggests a neutron superfluid interior surrounded and threaded by a charged component tied to the star's magnetic field. The pulsar's deceleration torque forces the charged component to corotate with the field but does not directly affect the neutral superfluid. On average, then, this latter component is always rotating faster than the exterior, and the frictional coupling between the two components will dissipate some of the star's rotational kinetic energy as heat. Greenstein³⁹, building on the work of Ruderman and Sutherland⁴⁰ who demonstrated that a deceleration of differentially rotating superfluid may not tangle the superfluid vortex lines, has generated a self-consistent model for the temperature of a neutron star as a function of time which includes frictional heating. More recent work⁴¹, including a careful treatment of relevant neutron scattering processes (by phonons, electrons and impurities), predicts that the temperature of a $0.5 M_\odot$ neutron star will remain above 3.5×10^5 K for 10^7 yr. More massive stars maintain even higher temperatures (for $M = 1.5 M_\odot$, $T \sim 5 \times 10^5$ K).

Independent lines of reasoning⁴²⁻⁴⁵ lead us to believe that virtually all radio pulsars are $< 10^7$ yr old. Figure 1 indicates then that, by using the Einstein Observatory, we may put the frictional heating results to a stringent observational test. The fraction of the stellar material in the superfluid state and thus the predicted temperatures are a strong function of stellar mass. If we accept the theory and yet detect no sources, the results may be interpreted as an upper limit on the masses of the various neutron stars involved. Alternatively, temperature upper limits below those predicted will provide information on neutron star structure. For example, the assumption that the superfluid vortex lines do not pin to nuclei in the crust⁴⁶ may have to be relaxed, implying that several other interesting phenomena must be considered⁴⁷⁻⁴⁹. Another possibility⁴¹ is that neutron stars have larger radii than those derived from any of the currently employed equations of state. Note that, in this regard, if one measures both the luminosity and the temperature (by fitting a black body spectrum) of a neutron star, the radius—a critically important parameter for distinguishing among stellar models—is uniquely determined.

Starquakes: In addition to the potential thermal energy stored in the differentially rotating neutron star, a large amount of elastic and gravitational energy resides in the solid outer crust. The crust is distorted into an oblate spheroid by its rapid rotation. As the rotation rate decreases, the star tries to readjust, but the

immense strength of the crustal material (shear modulus $\mu \sim 10^{29}$ dyn cm⁻²) resists, until the stress exceeds a critical value and the crust cracks. The resulting starquake was first proposed by Ruderman⁵⁰ to account for the sudden speed-up of the Vela pulsar. Although the magnitude of these 'glitches' ($\Delta P/P \sim 10^{-6}$) coupled with their roughly triennial recurrence casts serious doubt on this application of the starquake theory, it has been shown that erratic behaviour is a hallmark of pulsar rotation rates. The Crab pulsar has undergone at least two such discrete spin-ups (although of a much smaller magnitude: $\Delta P/P \sim 10^{-9}$), an older pulsar, PSR1641-45, has experienced a Vela-sized glitch, and a 'restless' wandering of rotation rates (often designated as 'timing noise') has been found in a large number of other objects (refs 51 and 52 summarise recent work). A starquake model may well be applicable to many of these phenomena⁵³.

The energy released in a typical starquake is of the order of 10^{40} – 10^{41} erg (ref. 54). In the simple two-component picture described above, one can calculate the interval between such quakes t_{sq} from the measured values of the star's angular frequency Ω , frequency derivative $\dot{\Omega}$, change in frequency as a result of the glitch $\Delta\Omega$, and several parameters which describe the structural properties of the star^{54,55}:

$$t_{sq} = \left(\frac{2A^2}{BI} \right) \left(\frac{\Delta\Omega}{\Omega^2 \dot{\Omega}} \right) \quad (9)$$

A is the gravitational energy stored in the star by virtue of its rotation, B measures the elastic energy stored in the solid crust due to its distortion, and I is the star's moment of inertia. While these parameters are quite sensitive to assumptions about the stellar mass and crustal properties, expected time scales range from $\sim 10^4$ yr for a $1.5 M_\odot$ star to a few days for stars one-tenth as massive⁵⁵. The observed interval for both the Vela and Crab pulsars is a few years. For older pulsars, only one large glitch has occurred in ~ 200 pulsar-years of accumulated data, although events with $\Delta P/P \sim 10^{-10}$ – 10^{-12} may be occurring frequently (hours to weeks) and manifesting themselves as timing noise⁵³.

This seismic activity results in an average rate of energy input to the neutron star $L_{sq} \sim E_{sq} t_{sq}^{-1}$. If a large fraction of this energy is ultimately radiated away as thermal photons, the time-averaged luminosity will be $\sim 10^{33}$ erg s⁻¹ for both Crab-type glitches and the more frequent, smaller quakes which result in timing noise. Since the thermal time constant for the star is long relative to t_{sq} , this process could maintain the stellar surface at temperatures of $\sim 10^6$ K for as long as it operates. Many (~ 50) nearby pulsars (see Fig. 1) would then be observable with the Einstein Observatory. Greenstein⁵⁶ has noted that the deposition of energy in a neutron star following a quake may trigger a thermal instability. By extracting energy from the more rapidly rotating interior, the star's temperature may rise significantly, rendering even more distant objects easily observable for some time after the event.

Accretion: It is beyond the scope of this article to discuss the thermal properties of neutron stars in binary X-ray systems where the dominant source of the observed radiation is material accreted from a normal stellar companion. However, we include a note on temperatures expected from an isolated star accreting interstellar material. If current estimates of the pulsar birthrate ($\sim 10^{-1}$ yr⁻¹) (ref. 42) are even approximately correct and if this rate has persisted throughout most of the life of the Galaxy, there are at present a large number of neutron stars ($\sim 10^9$ or $\sim 1\%$ of the total galactic mass) which are no longer active pulsars. The implied space density is such that the nearest object is expected to be ~ 10 pc from the Sun. The accretion rate $\dot{M}$ for such a star with mass M travelling at velocity v through the interstellar medium of density ρ was first given by Bondi and Hoyle⁵⁷:

$$\dot{M} = 3.73 \times 10^8 \left(\frac{\rho}{1 \text{ cm}^{-3}} \right) \left(\frac{M}{M_\odot} \right)^2 \left(\frac{v}{100 \text{ km s}^{-1}} \right)^{-3} \quad (10)$$

for spherically symmetric accretion. (The very old, slowly rotating stars under consideration here will most likely have little or

Table 3 IPC counts from pulsar polar caps at distance D from Earth

Luminosity (erg s ⁻¹)	T (10^6 K)	Counts*			
		$D = 0.1$ kpc	0.3 kpc	1 kpc	3 kpc
10^{28}	0.36	1.5	0.015	—	—
10^{29}	0.65	56	0.68	0.0024	—
10^{30}	1.24	720	26	0.68	0.011
10^{31}	2.0	6,600	470	22	0.74
10^{32}	3.6	80,000	7,600	480	28

* Counts observable in a 10^4 -s IPC pointing assuming an area for the polar cap of 10^{10} cm² and an interstellar neutral hydrogen density of 1 cm⁻³.

Table 4 Hot neutron stars in supernova remnants

Name	Age (yr)	D (kpc)	$R_{\max}^*$ (arcmin)	T (10^6 K)	Ref.
Cas A	~300	2.8	0.4	<1.5	71
Kepler	375	8.0†	0.2	<2.1	Present work
Tycho	407	3.0†	0.5	<1.8	Present work
Crab	925	2.0	1.6	<3.0	10
SN1006	973	1.0	3.3	<1.0	F. D. Seward and J. P. Pye (personal communication)
RCW86 (AD185)	1,794	2.5†	2.5	<1.5	Present work
W28	3,400	2.3‡	5.0	<1.5	Present work
G350.0	~10 ⁴	4.0‡	7.0	<1.5	Present work
-18					
G22.7-0.2	~10 ⁴	4.8‡	7.0	<1.8	Present work
Vela	~10 ⁴	0.4	1.4	~1.5	72

* Distance from SNR centre assuming a transverse velocity of 1,000 km s⁻¹.

† Ref. 69.

‡ Ref. 70.

no relativistic outflow to resist the accretion, although a significant remnant magnetic field may channel the material onto the polar cap regions.) Measured pulsar velocities range from 20 to 500 km s⁻¹ (refs 58–62). Adopting a characteristic conversion efficiency of rest mass energy of the accreted material to X rays of 0.1, we find a luminosity of 4.5×10^{29} erg s⁻¹ for a $1.3 M_{\odot}$ neutron star travelling at 50 km s⁻¹ through a medium with $n_H = 1$ cm⁻³. Such a star would be detectable to a distance of ~50 pc and might be distinguished from other background X-ray sources without optical counterparts by its large proper motion of ~1 arc s yr⁻¹. Stars passing through regions of much higher density (molecular clouds with $n_H \sim 10^6$) will have proportionally higher luminosities, although the increased absorption in such a region will probably render them undetectable.

Thermal dissipation—general considerations: Greenstein and McClintock³ pointed out that one might expect heating of neutron stars on general grounds from thermal dissipation of rotational energy. We consider here one circumstance under which such dissipation might take place, and then look at the size of the dissipation effects which could be measured in radio pulsars using the Einstein Observatory.

From the expressions for the rotational energy ($E = \frac{1}{2} I \Omega^2$) and angular momentum ($J = I \Omega$) of a rigid body, we have

$$\Delta E = \Omega \Delta J \quad (11)$$

This specifies a relationship between the energy lost and angular momentum lost by a spinning neutron star as it slows down. Now a photon of energy $E_p = \hbar \omega$ emitted tangentially from the light cylinder (at radius $r = c/\Omega$ from the neutron star) will carry off an amount of angular momentum J_p given by

$$J_p = \frac{\hbar \omega}{c} r = \frac{\hbar \omega}{\Omega} \quad (12)$$

so that

$$E_p = \Omega J_p \quad (13)$$

that is, radiation from the light cylinder carries off just the right amount of energy per unit angular momentum to satisfy equation (11); there is none left to heat the star. However, this situation is idealised in that it assumes corotation of the neutron star magnetosphere. In some circumstances (when $\mathbf{E} \cdot \mathbf{B} \neq 0$ along the field lines), Ω in fact will decrease outwards, causing the angular velocity Ω_{lc} at the light cylinder to be less than the neutron star angular velocity Ω . In this case, we have

$$E_p = \Omega_{lc} J_p < \Omega J_p \quad (14)$$

and the photons do not carry off sufficient energy per unit angular momentum; there is an excess $E_p - \Omega J_p$ per photon which is available to heat the star.

We can characterise such thermal dissipation from a neutron star in terms of the ratio $\eta = L_x/\dot{E}$ of the (thermal) X-ray luminosity to the rotational energy loss rate. Here η is not an efficiency in the usual sense (it could greatly exceed unity, for example, in the case of a hot young star). However, for old neutron stars which have had sufficient time to cool, η does provide a measure of (or upper limit on) the thermal dissipation of rotational energy.

For pulsars with measured period derivatives, one can calculate $\dot{E}$ using theoretical estimates of the moment of inertia²⁶. The results generally lie in the range 10^{31} – 10^{33} erg s⁻¹. Figure 1 shows that within ~100 pc, neutron star temperatures as low as 250,000 K, corresponding to luminosities of $\sim 10^{31}$ erg s⁻¹, can be detected with the IPC. Thus in several cases one could establish limits to η of $\lesssim 1\%$. That is, a fairly small fractional thermal dissipation of the rotational energy is, in principle, detectable. Such observations can therefore be expected to provide important constraints for the construction of pulsar models.

Observations

Past results: The observational situation regarding thermal emission from neutron stars has not been as rich in scope or detail as the theoretical work described above. The first attempt to measure the temperature of a neutron star was carried out in 1963 (before the discovery of pulsars) when a group from the Naval Research Laboratory observed a lunar occultation of the Crab Nebula with a rocket-borne X-ray detector⁶³. The result showed that the bulk of the X-radiation from the Crab came from a spatially extended source, implying that the temperature of any associated hot neutron star must be $\lesssim 10^7$ K. During the next series of Crab occultations, 11 yr later, two groups flew improved detectors and set limits to the temperature of the now-confirmed neutron star in the Nebula of 4.7×10^6 K and $\sim 3 \times 10^6$ K, respectively^{10,17}. Poor spatial resolution has meant that observations of other SNR with both rocket and satellite experiments have failed to yield significant ($T < 10^7$ K) upper limits for any other young objects. Limits on the temperatures of older neutron stars (radio pulsars) have been calculated using data from the Uhuru all-sky survey⁵; the results for the nearer pulsars ($D < 500$ pc) lie in the range 2.0 to 3.0×10^6 K, far above most of the predictions summarised above. Similar studies using data from Copernicus⁶⁴ and ANS⁶⁵ have yielded slightly higher limits. A recent optical spectrographic search of several historical SNR⁶⁶ showed no evidence of peculiar objects in the central regions of the nebulae, but the limiting magnitude of the survey was $m_{pg} 18.5$ (see Table 1). Finally, the best limits on neutron star temperatures recorded before this year were obtained with the extreme UV telescope aboard the Apollo-Soyuz mission⁶⁷. The results for three nearby pulsars ranged from 0.5 to 5×10^6 K but are extremely sensitive to the values assumed for the photoelectric opacity of the interstellar gas.

The Einstein Programme: The observing program of the Einstein Observatory calls for observations of over 50 SNR and 35 radio pulsars. All the historical SNR will be studied extensively with both the IPC and the high resolution imager (HRI—spatial resolution of 5 arc s)¹⁸; over a dozen older remnants within 3 kpc will also be examined closely for hot neutron star remnants. The pulsar survey includes many objects selected on the basis of short periods, large values of B_{12}/P^2 , and large amplitude timing noise to provide the most stringent tests possible for the various theories of pulsar emission mechanisms and neutron star structure discussed above. We have also included an unbiased survey of all objects within 350 pc of the Earth. We present below the first results of these observations.

Supernova remnants: Table 4 lists results for 10 of the SNR observed with the Einstein Observatory. The objects are ordered by age with the six historical remnants heading the list; for the remaining objects we have adopted ages determined by application of the standard blast wave (Sedov) model to the X-ray data⁶⁸. The distances adopted are published values and are consistent with those found from the measured hydrogen

Table 5 Temperature limits for radio pulsars

Name (PSR)	P (s)	$\dot{P}$ ($10^{-15} \text{ s s}^{-1}$)	D (pc)	Counts (0.1–5.0 keV)	$T_{n_0=0.3 \text{ cm}^{-3}}$ (10^6 K)	$T_{n_0=1.0 \text{ cm}^{-3}}$ (10^6 K)	T_{cap}^* (10^6 K)	L_{cap} ($10^{30} \text{ erg s}^{-1}$)
0031–07	0.943	0.40	440	26	0.4	0.7	2.4	5.5
0149–16	0.833	—	560	19	0.5	0.9	2.7	9.8
1529+28	1.125	—	640	16	0.5	0.9	2.9	9.6
1642–03	0.388	1.8	160	18	0.3	0.35	1.2	0.8
1706–16	0.653	6.4	160	29	0.3	0.4	1.4	0.9
1952+29	0.427	0.002	240	31	0.3	0.4	1.5	1.8
2327–20	1.644	—	320	17	0.4	0.5	2.2	2.2

* Polar cap areas have been calculated using equation (5) and assuming a radius of 16 km. A hydrogen density of 1 cm^{-3} has been adopted. Changing either of these assumptions by a factor of 2 changes the derived temperature limits by less than 30%. All temperatures and luminosities listed are 3σ upper limits.

column density derived from the X-ray spectra assuming a reasonable range of average interstellar values of n_{H} . (For example, the radio absorption spectrum⁷³ and X-ray determined column density of Tycho's remnant imply a neutral hydrogen density of $\sim 0.5 \text{ cm}^{-3}$ for the adopted distance of 3 kpc.) Table 4 also lists the distance in arc min from the centre of the explosion within which a neutron star would be found if it had a transverse velocity of $<1,000 \text{ km s}^{-1}$. (The mean velocity of radio pulsars derived from proper motion measurements is $\sim 150 \text{ km s}^{-1}$ and the highest observed velocity from a sample of 25 objects is 500 km s^{-1} (ref. 62).) The temperature upper limits for a point source of X rays in these remnants is also given. For the Columbia IPC observations, we have determined a 3σ upper limit to a point source counting rate by sliding a 2 arc min radius circular detection cell through the allowed region and determining the local background level from a concentric annulus with inner and outer radii of 2 and 3 arc min, respectively. This value is then converted to a temperature limit following the procedure and adopting the assumptions outlined above. For Kepler's remnant, preliminary HRI data have recently become available, and we have used these with a 12 arc s search cell to set an upper limit. Similar procedures were used for the remaining observations (see cited refs for details).

Of the six historical SNR, only the Crab is known to contain a remnant neutron star. The analysis of Einstein data for this source is not yet complete, so its temperature limit remains at the value from the lunar occultation experiments. For all of the remaining historical objects, the measured temperature limits fall below the lower limits predicted by nearly all current work on the cooling of neutron stars.

Among the four older sources, only Vela was known to contain a neutron star, and again the situation remains unchanged. The limits on the remaining three sources are also either below or just consistent with the lower bounds derived from standard cooling theories. However, a point source has tentatively been detected at the position of the Vela pulsar⁷³, and the derived temperature of $1.5 \times 10^6 \text{ K}$ is consistent with that expected for a 10^4 yr old star. The observation is somewhat complicated by the fact that the point source is surrounded by a small (1 arc min) region of diffuse emission and the interpretation of the central cusp of this nebula as thermal radiation from the neutron star should be treated with caution.

Radio pulsars: Seven nearby radio pulsars have been observed in the Columbia survey; the sources are listed in Table 5. No sources have been detected at the nominal pulsar positions. Upper limits to the IPC counting rates and the resultant temperature limits for thermal stellar emission were derived as discussed above. Limits on polar cap temperatures and total luminosities are also given. All of these values are typically an order of magnitude below those previously available and provide interesting constraints on several theoretical problems.

Discussion

An observational determination of the cooling rate for young neutron stars is a fundamental input to models of neutron star structure. Such a determination is within the capabilities of the

Einstein Observatory. The results on the historical SNR imply either that many contain no neutron star remnant, or, in the context of our current understanding of cooling theory, pion condensation may need to be invoked in order to enhance the cooling rates. In view of the importance of the latter possibility, we wish to encourage thorough theoretical studies of neutron star cooling to verify this conclusion.

The option that some supernovae leave no neutron star remnant also holds important implications. While supernova models which produce neutron stars have recently been constructed, the alternatives of a black hole remnant or an explosion which dissipates the entire star will have to be explored. A more immediate problem, however, concerns the pulsar birthrate. The current best estimates for the galactic supernova rate is one per 30–50 yr. The pulsar birthrate has been estimated to be one per 5 yr (ref. 42). This latter value is sensitive to several assumptions, such as the electron density of the interstellar medium which sets the pulsar distance scale and the 'beaming factor' which specifies what fraction of the total population is observable. Taylor and Manchester⁴² assume a pencil beam radiation pattern for pulsars and adopt a beaming factor of 20%. If, in fact, the pattern were closer to a fan beam such that essentially all pulsars were observable, much of the above-noted discrepancy would disappear. The fact that only one of the five supernovae of the past 1,000 yr has left behind an observable radio pulsar has been used as an argument in favour of the pencil beam model. However, the lack of an X-ray point source in the remaining objects may suggest that only a small fraction of SN events leave neutron star remnants and, thus, even if a fan beam model is adopted, the discrepancy between supernova and pulsar production rates will remain large. Some alternative production mechanism for neutron stars, such as accretion pushing a white dwarf over the Chandrasekhar limit, may have to be considered.

While the observation of newly formed neutron stars in supernova remnants promises to yield valuable information for our understanding of neutron star matter, X-ray data on the thermal properties of radio pulsars will provide constraints on models for many aspects of pulsar behaviour, including crust-core coupling, starquakes, pulsar emission mechanisms, and so on. Our preliminary results illustrate the model testing that such observations may provide, even without the actual detection of thermal radiation. In testing polar cap emission models we have calculated the expected luminosities based on equations (5)–(8); for sources without measured values of $\dot{P}$ (and, thus, no computed value for the field strength), we have assumed $B_{12} = 1$. While none of these objects is expected to exhibit the intense radiation characteristic of the γ -ray emitting pulsars, three of the sources, PSR1642–03, PSR1706–16 and PSR1952+29 constrain the value of ε in the inner gap theory²⁹ to be $\varepsilon \leq 0.1$ (the limits are still above the predictions from the minimum flux polar cap model of Arons and Scharlemann³³). Using the frictional heating model⁴⁰, we can set mass limits on the three sources with temperatures of $<300,000 \text{ K}$ of $<0.5 M_{\odot}$, significantly below the measured masses of neutron stars in binary systems. Alternatively, in a model-independent way, we

can say that for PSR1642–03 and PSR1706–16, the efficiency for conversion of rotational to thermal energy is <1%. More results will make all these constraints much more definitive as we open a new window for observational studies of neutron stars

through the search for their thermal X-ray emission.

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ARTICLES

Reactivation of basement faults and crustal shortening in orogenic belts

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During the early evolution of some fold mountain belts old listric normal faults in a stretched and thinned basement beneath the sedimentary column may become reactivated as thrust faults. The reversal of motion on these faults allows considerable shortening to occur without subduction or excessive thickening of continental crust. This reactivation hypothesis is supported by the present-day seismicity of the Zagros collision zone and the structure of sedimentary basins.

PLATE tectonics has been very successful in describing the overall deformation and kinematics of the large ocean basins but there is no comparably simple description to account for the general behaviour of the continents.

Recent studies of continental deformation have indicated some of the notable differences between oceanic and continental behaviour. The major strike-slip faults in the Alpine-Himalayan Belt^{1–3} seem to slide large wedges of continental material away from zones of collision, thereby avoiding the excessive thickening of continental crust. Such large strike-slip features are easily visible on satellite photographs, and their spectacular nature, together with various models proposed to explain their large-scale significance^{2–5}, have to some extent diverted attention from the large areas of the Alpine-Himalayan belt which are undergoing active compression or extension.

These studies show that present day continental deformation is not a simple phenomenon. The deformation was unlikely to be less complicated in the past, and models for the evolution of old

fold mountain belts based on oceanic compressive margins probably have limited value. In particular, little progress seems to have been made towards solving the classical space problem in orogenic belts: if the upper crust is shortened several tens of kilometres by folding and thrusting, what happens to the basement? Very often the sediments involved in foreland folding and thrusting are separated from their basement by a decoupling horizon, often of evaporites or shale, which provides a surface of décollement separating structures above from those below. Subduction has been discussed as a mechanism for removing continental crust^{6,7}, and it has been concluded that the buoyancy of continental material should hinder its subduction and lead to crustal thickening instead^{7,8}.

Sedimentary basins

Helwig⁹ pointed out how this space problem in shortened orogenic belts is greatly diminished if the basement underneath the folded belt is thin to start with. Subsequent shortening then

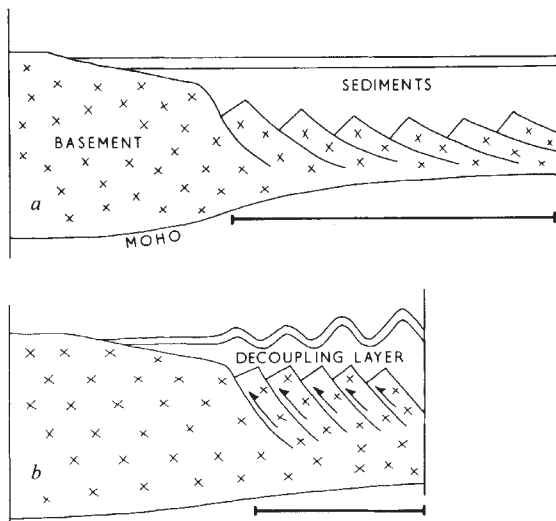


Fig. 1 Reactivation of basement faults. *a*, Sedimentary basin formed by deposition on a basement which has been stretched and thinned by listric normal faulting. *b*, When the basin contracts motion is reversed on the normal faults which are now used as thrusts. The sedimentary column takes up the shortening by folding.

thickens it, but not to an abnormal degree. McKenzie¹⁰ has since shown that the subsidence of continental sedimentary basins can be quantitatively described by a simple model in which the lithosphere is rapidly stretched and thinned, sinks quickly to maintain isostatic equilibrium, and then sinks more slowly as it cools in a manner similar to that described by the successful oceanic models¹¹. The initial stretching of the basement at shallow depths is thought to occur by listric normal faulting and thereafter stretching ceases and subsidence continues without further faulting. This model is supported by the crustal thickness and heat flow of the Aegean, which is still being stretched by normal faulting, and seems to account well for the subsidence history of the North Sea¹² and many of the intra-Carpathian basins¹³, all of which show an initial normal faulting episode followed by subsidence with no further faulting. Royden *et al.*¹⁴ have shown that the gross features of the same model will account for the stretching and subsidence preceding the break-up of a continent to form an Atlantic-type margin. It thus seems probable that thick sedimentary sequences generally form on thin extended basement which was stretched by normal faulting. Evidence for attenuated basement is best seen in seismic refraction profiles and has been demonstrated in the North Sea^{15,16} and Pannonian Basin¹³. The Aegean is in an earlier stage of development, with high heat flow¹⁷, thin crust^{18,19} and active normal faulting²⁰. Helwig's⁹ suggestion may, therefore, have some validity and the basement under the thick piles of sediments that make up fold mountain belts was probably thin before the onset of compression. The early stages in the development of a fold mountain belt following continental collision are now considered.

It is proposed that thick sediments were initially deposited on a subsiding basement which had been stretched by normal faulting (Fig. 1). The faulting was very probably of a low angle listric nature, spread over a region several tens of kilometres wide as observed in the Great Basin²¹. As stretching proceeded, an Atlantic-type margin was ultimately formed with thick sediments on the continental shelf overlying a thin basement. In the early stages of continental collision the basement takes up the shortening by reversing the sense of motion on the pre-existing normal faults (Fig. 1), which are now used as thrusts (reverse faults). The basement tends to return to its original thickness while the overlying sedimentary column, having been deposited on an extended basement, is forced to take up the shortening by folding. Thus in the early stages of collision the space problem is avoided without thrusting continental basement into the mantle or thickening it beyond its original (unstretched) state.

Seismicity of the Zagros mountains, Iran

The Zagros mountains of Iran provide an example of a young fold mountain belt currently shortening as a result of the collision between Arabia and Iran. The structure of the Zagros is very simple superficially, with a thick conformable sequence of Palaeozoic-Mesozoic-Tertiary shelf deposits warped into gentle folds in a single process in the latest Tertiary²²⁻²⁴. Long linear fold axes and seismic activity that is predominantly thrusting of the same orientation (refs 1, 25 and Fig. 2) both indicate a general NE-SW shortening. Although strike-slip faulting is present on the north-east front of the Zagros, especially in the north^{26,27}, it is not seen in the fault plane solutions of the folded belt (Fig. 2) and the relative motion between Arabia and Iran is normal to the strike of the belt.

The Folded Belt²² of the Zagros is seismically very active with earthquakes spread over an area ~300 km wide and with a distinct northeastern boundary roughly coincidental with the Zagros Thrust Line (or Main Zagros Reverse Fault, Fig. 2 and ref. 27), which in turn marks the edge of the Zagros sedimentary trough. These earthquakes are probably all shallower than 40 km (see ref. 28 for discussion) and there is no reliable evidence that they increase in depth towards the north-east margin of the Zagros. Consequently there is no evidence for seismic shortening on a single shallow-dipping plane, as is commonly assumed for areas of oceanic underthrusting. Fault plane solutions in the Zagros consistently show thrusting with comparatively high angle (40–50°) fault planes (ref. 29 and Figs 2 and 3). This is in marked contrast to the shallow angle (0–10°) thrusting which occurs in areas where oceanic underthrusting of continents is or was taking place, such as the Hellenic Arc, Makran and Eastern Himalaya²⁹. The seismic activity of the Zagros Fold Belt reflects high angle reverse faulting on a large number of faults distributed across the whole width of the belt.

Although microearthquake surveys in the Zagros have shown that small earthquakes do occur within the sedimentary column³⁰⁻³² it has been suggested that the largest shocks, which reach m_b 6.0–6.3, have focal depths of about 15 km, that is, beneath the sedimentary cover and in the crystalline basement. Berberian and others^{27,33} reached this conclusion from a study of damage distributions and because of the lack of surface faulting

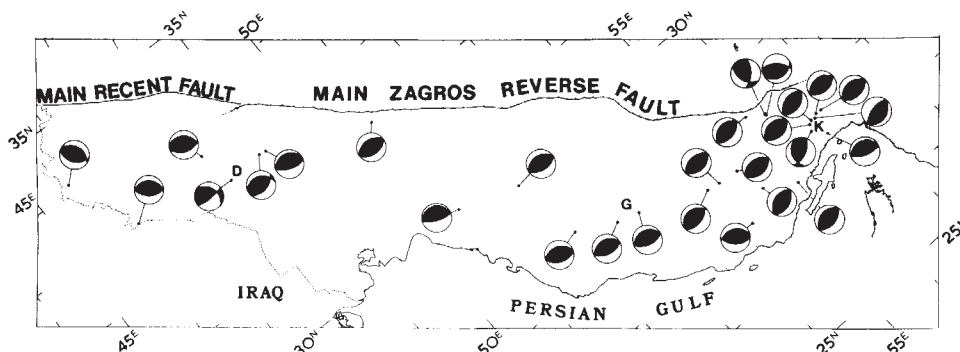


Fig. 2 Fault plane solutions from the Zagros from ref. 25, to illustrate the high angle thrusting in the Folded Belt south-west of the Main Zagros Reverse Fault (called the Zagros Thrust Line by Stocklin²⁴ and others). D, G and K mark the positions of the Dezful Embayment, Ghir and Khurgu earthquakes. Black quadrants are compressional.

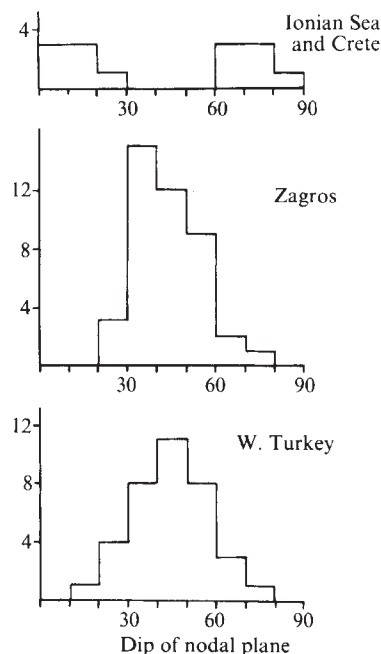


Fig. 3 Histogram to illustrate the high angle (40–50°) nature of the fault planes in the Zagros compared with those in the Ionian Sea and Crete, which are both part of the Hellenic arc where active subduction (intermediate depth earthquakes and andesitic volcanism) is taking place and thrusting is on shallow dipping (0–10°) fault planes. The Zagros histogram is similar to that of western Turkey where active extension by normal faulting is occurring. Dips are taken from the fault plane solutions in refs 1, 20, 25 and are mostly well controlled in the Zagros, even though the strikes may not be.

in the Zagros after major earthquakes. At the base of the sedimentary column is the thick infracambrian Hormuz Salt Formation, and additional salt is present in the Jurassic and especially in the Tertiary Gachsaran Formation²³. Any basement faulting is unlikely to propagate to the surface through these ductile horizons. It is also unlikely that shocks the size of the 1972 Ghor earthquake ($m_b = 6.0$), with a source dimension of about 30 km (refs 34, 35), could be contained within 6 km of sediment thickness without showing surface faulting. The most convincing evidence of basement faulting comes from detailed examination of the long period teleseismic waveforms from Zagros earthquakes. Shocks in the magnitude range m_b 5.5–6.0 are common in the Zagros, and their far-field long period radiation is usually very simple. In particular, the first cycle of the waveform can easily be modelled and is very dependent on focal depth. At long periods crustal structure has little effect on the first cycle of the waveform if the source is above the Moho, and earthquakes of this size can be modelled effectively by a single source as they rarely exhibit multiple rupture characteristics. Studies of the waveforms³⁶ demonstrate that in three areas of the Zagros—the Dezful Embayment, Ghor and Khurgu (Fig. 2)—earthquakes occurred at depths of 12–15 km. In each case this is below the probable thickness of the sediments^{37–39}. An example of this waveform modelling is shown in Figs 4 and 5. There is, therefore, the strong probability that the basement beneath the Zagros Fold Belt is deforming by high angle (40–50°) reverse faulting.

Evolution of the Zagros

There is much evidence that, during the Mesozoic and Tertiary, what is now the folded belt of the Zagros was a subsiding continental margin^{23,24,40,41}. As the basement was almost certainly cut by normal faults which caused its extension before

the onset of subsidence and sediment deposition in the Permo-Triassic, the present day reverse faulting is probably happening on the old normal fault surfaces which have been reactivated. This would explain the high (40–50°) rather than low (0–10°) angle nature of the fault dips, and the similarity of the dips of fault planes in western Turkey (currently extending by normal faulting) with those in the Zagros (Fig. 3). Reactivation of old faults is a common and important phenomenon⁴² and the reactivation of old normal basement faults as thrusts has been reported from Chile⁴³. The possibility of reactivation in the Zagros was alluded to by Falcon²², and Stocklin²⁴ proposed that the Zagros Thrust Line itself was a reactivation of an old Precambrian rift boundary which controlled the distribution of the Hormuz salt. The north-west trend of the Zagros is found in the Precambrian of the north-east Arabian shield²⁴. The same trend is evident in the sediment isopachs of the northern Zagros (Lurestan and Kuhzistan) throughout the Mesozoic^{44,45}.

An objection to this suggested basement involvement is the evidence from seismic reflection lines in old fold mountain belts, such as the eastern Rockies^{46,47}, which shows apparently undeformed Precambrian shield dipping gently beneath the foreland folds. However, it is likely that in these older orogenic belts the amount of shortening involved is considerably greater (~160 km, or 50%, in the Canadian Rockies^{46,47} than that which has taken place so far in the Zagros Fold Belt (about 20–50 km, or 20%, ref. 22) which is in a much younger state of development. The contraction mechanism proposed here will presumably work until the reverse motion on old normal faults restores the basement at least to its original thickness. Thereafter the basement will either become thicker than its pre-stretched state or be thrust into the mantle. It is probable that the sediments of the folded belt of the Zagros are still almost above the original basement on which they were deposited. As shortening continues, the folded cover will start to migrate south-west over the undeformed Arabian shield, separated from its basement by the décollement at the level of the Hormuz salt. The foreland folds of the Rockies, Appalachians and Jura are no longer above their original basement which was left behind in the internal zones. Those orogenic belts which are known to have thick crust

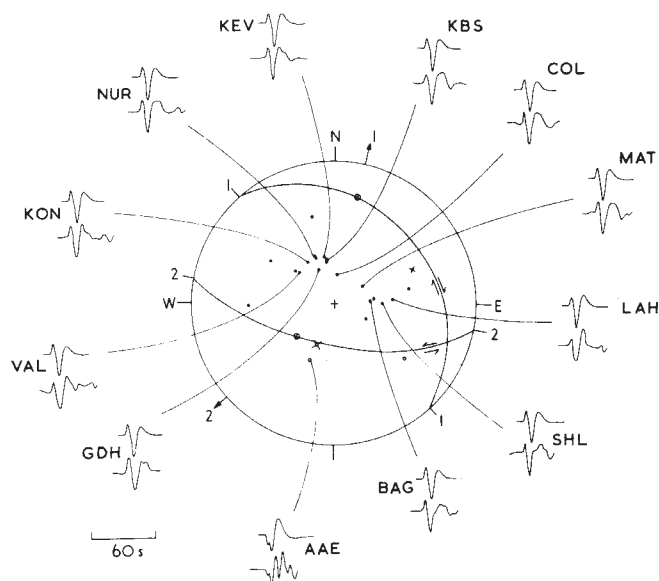


Fig. 4 Waveforms from a shock in the Dezful Embayment (Fig. 2) on 5 June 1977. The fault plane solution shows predominantly thrusting. Filled symbols are compressional first motions, open are dilatational and crossed are nodal. Synthetic waveforms matched the observed well at a focal depth of 12 ± 3 km (Fig. 5). For each station the observed seismogram is shown beneath the synthetic. Note particularly the kink in the downward pulse at AAE which is the pP arrival. Numbered arrows mark the slip vectors corresponding to planes 1 and 2.

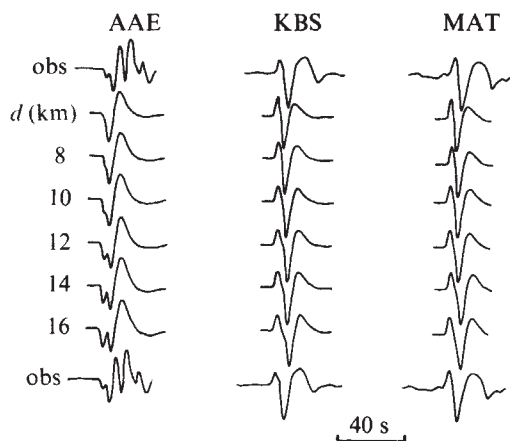


Fig. 5 Variation of waveform with depth (d) at selected stations in Fig. 4.

are all older and in more advanced states of development than the Zagros and have involved more shortening. An interesting prediction of the scheme proposed here is that, in the initial stages of continental collision, the basement under the orogenic belt should be thinner, not thicker than that of its neighbouring craton. At present no adequate refraction data from the Zagros is available to test this.

Conclusions

The origin of a young folded belt proposed here accounts well for the present-day seismicity and deformation of the Zagros. Such an origin involves the reactivation as thrusts of listric normal faults in a stretched and attenuated basement on which thick sediments of the Atlantic-margin type were deposited, and does not necessitate either abnormal thickening or subduction of continental crust in the early stages of continental collision. How long this process can continue is not clear. It is unlikely to account for all the features of older orogenic belts which have involved considerably greater shortening. However, even if operative in only the early stages of collision, it greatly reduces the space problem encountered in palinspastic reconstructions of orogenic belts⁴⁹.

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Lateral mobility in reconstituted membranes—comparisons with diffusion in polymers

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The diffusion coefficients (D) of lipopolysaccharide, phospholipid, and Escherichia coli matrix protein were determined in reconstituted multibilayer membranes. Over a range of protein concentration of 0–60% by weight, D for lipopolysaccharide decreased 10-fold, whereas D for phospholipid remained essentially constant. The diffusion coefficient of matrix protein at a concentration of 50% was $\leq 10^{-12} \text{ cm}^2 \text{ s}^{-1}$. These results are discussed in terms of a model for diffusion in polymeric networks.

THE fluid dynamics of biological membranes has been clearly established with the emergence of physical techniques to determine quantitatively the diffusion characteristics of membrane components^{1–4}. Measurements of diffusion constants by fluorescence photobleaching methods have provided evidence for the lateral mobility, over micrometre distances, of

lipids, glycolipids and integral membrane proteins in eukaryotic cells (see, for example, refs 5–10). In addition, measurements of lipid and glycolipid diffusion have been obtained in phospholipid model membrane systems devoid of proteins^{11,12}. To extend these results and provide a systematic approach to an understanding of membrane diffusion, we have developed a reconstituted membrane system containing an integral membrane protein. We believe that the techniques used can be

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extended to reconstitute other membrane systems for mobility investigations.

The reconstituted system to be described is composed of matrix protein, phospholipids and lipopolysaccharide isolated from the outer membrane of Gram-negative bacteria, *Escherichia coli* and *Salmonella typhimurium*. The outer membrane contains approximately 20–25% phospholipid, 30% lipopolysaccharide and 45–50% protein¹³. The matrix protein of *E. coli* (molecular weight of monomer, 36,000) is a transmembrane protein which forms hydrophilic pores, allowing low molecular weight carbohydrates, amino acids and ions through the permeability barrier of the outer membrane¹³. In association with lipopolysaccharide, it forms a phage receptor¹⁴. Electron microscopy of *E. coli* membrane preparations has demonstrated that matrix protein forms a highly periodic monolayer consisting of homopolymer trimers interacting to cover the peptidoglycan surface^{15,16}. Reconstituted in vesicles, it retains its pore function¹⁷, can inactivate phage and forms voltage-dependent aqueous channels in planar lipid bilayers¹⁸. Lipopolysaccharide is an amphipathic glycolipid that is only found in the outer membrane of Gram-negative bacteria and seems to be a major contributor to the permeability barrier in these organisms¹⁹.

The experiments to be described suggest that diffusion of membrane components in the plane of the membrane is analogous to the diffusion of non-electrolyte molecules in a polymer network. Lieb and Stein have presented such a comparison for diffusion through biological membranes^{20,21}. Our results should enable us to extend their observations and make some

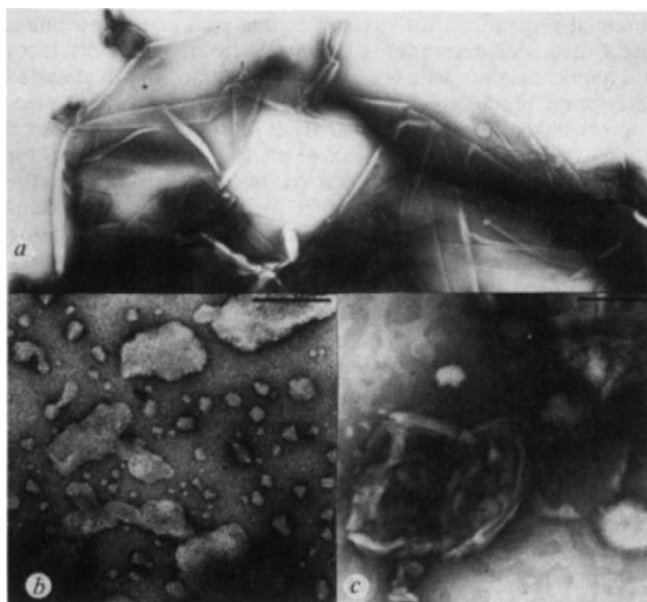


Fig. 2 Electron microscopy of vesicle preparations. Vesicles composed of phospholipid/matrix protein/lipopolysaccharide at ratios of 1:1.3:1 (a), 1:4:1 (b), and 1:0:1 (c), were negatively stained with 2% uranyl acetate on Formvar-coated specimen screens. Samples were examined with a Hitachi 11 E electron microscope at 75 kV. Scale bar, 250 nm.

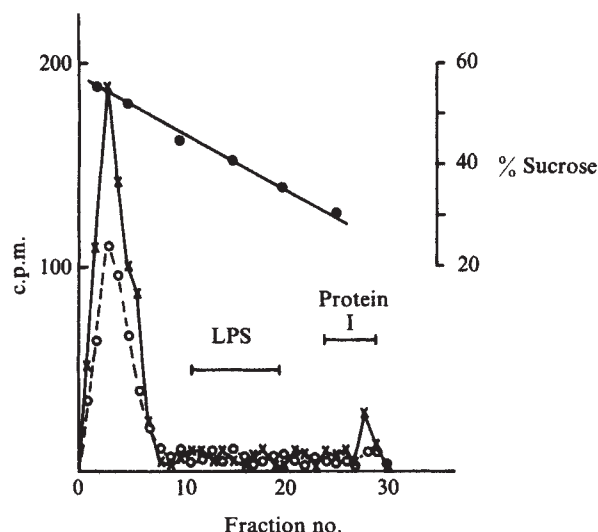


Fig. 1 Sucrose density gradient of reconstituted vesicle preparation (1:1.3:1, phospholipid/protein/lipopolysaccharide). Vesicles were prepared by the method of Nakae¹⁷ with minor modifications. Phospholipids were isolated from *E. coli* K12 grown at 37 °C by the method of Raetz and Kennedy³⁴. Lipopolysaccharide was derived from strain G30 of *Salmonella typhimurium* by the method of Galanos *et al.*³⁵. Further purification was achieved by electrodialysis³⁶. Matrix protein was isolated by the procedure of Hindennach and Henning³⁷. The protein was then applied to a P-150 column (2.5 × 40 cm) water jacketed and eluted at 37 °C with 10 mM Tris, 2% SDS, 0.5 M NaCl and 5 mM EDTA to remove bound lipopolysaccharide. The protein was solubilised by slow dialysis of an 8 M urea-protein solution against 10 mM HEPES, pH 7.5. Protein concentrations were monitored by modified Lowry procedure and spectrophotometrically using an extinction coefficient, $A_{280}^{1\%} = 1.3$. Following an overnight incubation procedure¹⁷, 20 mM disodium EDTA was added to the vesicle preparations, which were then placed on ice for 10 min and dialysed overnight at room temperature against 101 of distilled water. Vesicles (50 μ l) containing ³H-labelled phospholipid (×) and ¹⁴C-labelled lipopolysaccharide (○) were resuspended in 1 ml of 25% sucrose (w/w) in 10 mM HEPES, pH 7.5, and layered onto a sucrose gradient containing 2.1 ml each of 50, 45, 40, 35 and 30% sucrose (w/w) over a cushion (0.5 ml) of 55% sucrose. All sucrose solutions contained 10 mM HEPES, pH 7.5. Centrifugation was carried out in a Beckman SW 41 rotor at 36,000 r.p.m. for 18 h at 4 °C. Gradients were fractionated by puncturing the bottom of the centrifuge tube and collecting drops. Protein was assayed by fluorescamine labelling and fluorimetric analysis of each gradient fraction.

general statements concerning two-dimensional diffusion in membranes.

Characterisation of vesicles and multibilayers

Isopycnic sucrose gradient centrifugation of vesicles (Fig. 1) showed a single homogeneous peak containing phospholipid lipopolysaccharide and protein. Figure 2a is a negative-stained preparation of the vesicles (phospholipid/protein/lipopolysaccharide, 1:1.3:1) before multibilayer formation. At protein concentrations of >20% by weight, long sausage-shaped sacks (~1 μ m long) were the predominant vesicle species with a subpopulation of smaller vesicular structures which were probably intermediates between small unilamellar vesicles and the larger cylindrical vesicles. Figure 2b is a negative-stained preparation of vesicles (1:4:1) at higher resolution demonstrating ordered membranous lattice structures. These structures do not appear in protein-free vesicles (Fig. 2c). Comparison of Fig. 2b and c provides evidence that (1) the matrix protein is physically integrated into the membrane, as also indicated by the sucrose density gradients, and (2) the protein is uniformly distributed over the surface of the membrane. Figure 3a, b shows thin sections of multibilayer preparations from such vesicles. These sections indicate that stacked bilayers are formed from the vesicles and that these bilayers extend over long distances (approximately 1–2 μ m in the figure).

Determination of diffusion coefficients

Lateral diffusion coefficients of fluorescently labelled phospholipid, lipopolysaccharide and protein were determined in 'fluorescence redistribution after photobleaching' experiments. In this technique (also referred to as 'fluorescence photobleaching recovery'), translational transport in the plane of the membrane is characterised by the kinetics of fluorescence 'recovery', due to the spatial redistribution of fluorescently labelled molecules after an initial, localised photobleaching pulse. A new multi-point analysis was used in which the fluorescence redistribution is monitored by a focused laser beam sequentially positioned at each of several locations immediately adjacent to the bleached area. The spatial information thus

obtained provides sensitivity to possible systematic flow and a direct means of determining characteristic transport distances and hence absolute values of transport coefficients. A detailed description of the theory and experimental optics and electronics used in the multi-point analysis is presented elsewhere²². Briefly, a standard fluorescence microscope, equipped for incident light excitation, is used to focus the laser beam onto the sample and collect the fluorescence for detection by a thermoelectrically cooled photomultiplier tube. The precise orientation of the incident laser beam, and hence the location on the sample of the focused spot along a scan axis, is modulated by a servo-activated galvanometric optical scanning mirror. The laser intensity is switched between monitoring and bleaching levels, differing by a factor of 2×10^4 , by a combination of diaphragms, beam-splitters and an electronic shutter.

N-4-nitrobenzo-2-oxa-1,3-diazole (NBD) fluorescence was monitored with an incident wavelength of 4,765 Å and a combination of Leitz dichroic mirror TK510 and barrier filter K510. Rhodamine fluorescence was monitored with an incident wavelength of 5,309 Å and a combination of Leitz dichroic mirror TK580 and barrier filter K570. The membranes were examined before the photobleaching experiments with dark-field or phase-contrast transmitted light illumination. All photobleaching measurements were carried out with a $10\times/0.25$ NA dry achromat.

In most experiments, the fluorescence was monitored at three positions on a scan axis, with Δx , the position of the monitoring beam relative to the bleach position, equal to 0 and ± 3.0 µm. Experimental values of $F(\Delta x, t)$, the fluorescence intensity at a time t after the bleaching pulse, were fitted to the following form

$$F(\Delta x, t) = F(-) \{ [1 - \beta \alpha(t) \exp[-(\Delta x)^2/w^2(t)] - (1 - \beta) \alpha_0 \exp[-(\Delta x)^2/w_0^2]] \} \quad (1)$$

with

$$\alpha(t) = \alpha_0 / (1 + t/\tau_D) \quad (2)$$

$$w^2(t) = w_0^2 (1 + t/\tau_D) \quad (3)$$

where

$$\tau_D = w_0^2/4D \quad (4)$$

is the characteristic time for diffusion; $F(-)$ is the prebleach fluorescence intensity; α_0 is a constant ($0 < \alpha_0 < 1$) which characterises the extent of bleaching; β is the fractional recovery at infinite time; and w_0 is the $1/e$ radius of the assumed gaussian profile of the fluorescence scan at time zero. From each set of data, we computed values of β , τ_D , w_0 , and hence D .

Separate control experiments were carried out to check for the possible occurrence of dye-sensitised protein aggregation induced by intense laser irradiation. After the same irradiation doses that produced extensive protein cross-linking in red cell ghosts labelled with fluorescein-concanavalin A (ref. 23), we observed no evidence of protein aggregates on SDS-polyacrylamide gels of labelled vesicles containing 50% protein.

Diffusion of lipopolysaccharide, phospholipid and matrix protein

All diffusion measurements were carried out using a double label: *N*-4-nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine (NBD-PE) and rhodamine-labelled lipopolysaccharide or matrix protein. In each instance, recovery of NBD fluorescence was measured in tandem with measurement of rhodamine recovery on the same patch of reconstituted membrane. All measurements can therefore be related to a phospholipid mobility allowing accurate comparisons. Figure 4a shows a set of fluorescence recovery curves for rhodamine-labelled lipopolysaccharide in a multilayer membrane 1:1.3:1 by weight phospholipid/protein/lipopolysaccharide. The fluorescence was monitored at three equally spaced positions with a 3.0 µm separation. Note that the two curves measured at 3.0 µm on either side of centre are identical, consistent with an isotropic diffusive transport mechanism. The solid lines are

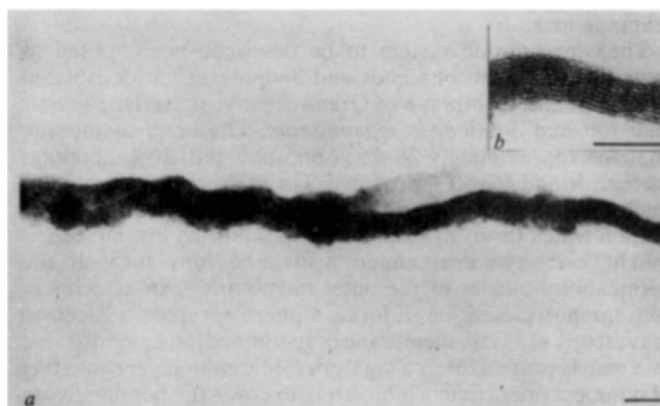


Fig. 3 *a, b*, Thin section of multibilayers prepared from vesicles (ratio 1:1.3:1). Multibilayers were prepared by using several techniques cited in Wu *et al.*¹². A 15-µl portion of the vesicle preparation was applied to a slide resting on a slide warmer set at 45 °C. After drying, another 15 µl was applied. This procedure was followed until 90 µl of vesicle preparation had been deposited on the slide. The slide warmer was then switched off and the slide allowed to reach room temperature. To rehydrate the membrane, a coverslip was sprayed with an aerosol containing 50 mM HEPES, pH 7.5, providing a uniform distribution of small droplets. The wetted side of the coverslip was then placed face down on the dried vesicle preparation. A Teflon cover was put over the coverslip and the slide placed between two aluminium blocks. A 25 lb lead brick was then placed on the top block for 20 s. Following pressing, the slide was removed and the coverslip edges sealed with melted paraffin. Samples to be prepared for thin section were sealed with paraffin on only two sides of the coverslip. The multibilayers were fixed in 2% glutaraldehyde and 0.1 M sodium cacodylate, pH 7.35, by placing pieces of filter paper over the sample and soaking the paper in fixative. The preparations were left overnight at 4 °C after changing the filter paper and then applying buffer. Post-fixation (1 h, 24 °C, 2×) was carried out with 2% OsO₄ using the filter paper technique of application. After a buffer rinse with filter paper the samples were dehydrated in series ethanol to 100%. During dehydration the coverslip floated off with the multibilayers remaining attached to it. The preparations were then infiltrated with three changes of Epon 812 (multibilayer side), embedded in Epon 812 and hardened at 60 °C for 2 days. Various areas were selected and then re-embedded to allow sectioning normal to the membrane surface. Thin sections (silver) were stained on the grid with 2% uranyl acetate followed by Reynold's lead citrate, 5 min each. Scale bar, 100 nm.

theoretical curves calculated according to equations (1)–(4), corresponding to $\beta = 0.80$, $\tau_D = 153$ s, $w_0^2 = 10.8$ µm² and $D = 1.76 \times 10^{-10}$ cm² s⁻¹. Figure 4b shows fluorescence recovery curves for NBD-PE and rhodamine-labelled lipopolysaccharide measured on a single patch of membrane, of the same composition as described above. For simplicity, only the fluorescence measured coincident with the bleaching positions is shown. For comparison, the fractional recoveries

$$f(t) = \frac{F(0, t) - F(0, 0)}{F(0, \infty) - F(0, 0)} \quad (5)$$

are plotted on a normalised time axis, t/w_0^2 . These data correspond to a value of $D_{LPS}/D_{PL} = 0.14$ (D_{LPS} and D_{PL} refer to the diffusion coefficients of lipopolysaccharide and phospholipid, respectively).

Figure 5 is a compilation of our results for NBD-PE and rhodaminated lipopolysaccharide. With the exception of the open points (see below), the data were derived from membrane preparations with a ratio of phospholipid to lipopolysaccharide of 1:1. At 24 °C, the mobilities of lipopolysaccharide and phospholipid are essentially the same in the absence of protein ($D_{LPS} \approx 1 \times 10^{-9}$ cm² s⁻¹; $D_{PL} \approx 1.5 \times 10^{-9}$ cm² s⁻¹) (Fig. 5a). The mobility of phospholipid in this system is about an order of magnitude slower than reported for model systems containing a single phospholipid species^{11,12}. Decreased phospholipid mobilities have already been noted in mixed lipid systems^{1,24}. The rate of phospholipid diffusion is practically independent of protein concentration. However, D_{LPS} decreases by a factor of 10 between 0 and 60% protein content.

The open points in Fig. 5 represent data from membranes with a phospholipid to lipopolysaccharide ratio of 12:1. These values

are in line with the others, suggesting that diffusion rates are primarily determined by the total protein concentration and not the individual ratios of phospholipid or lipopolysaccharide to protein. Note also that the diffusion coefficient for lipopolysaccharide is significantly greater at 37 °C than at 25 °C, whereas that for the phospholipid remains practically the same; $D_{LPS\ 37^\circ C}/D_{LPS\ 25^\circ C} \sim 2$, whereas $D_{PL\ 37^\circ C}/D_{PL\ 25^\circ C} \sim 1$ for 40% protein. Matrix protein at concentrations of 40% or 60% demonstrated extremely low diffusion coefficients at room temperature ($\leq 10^{-12}\text{ cm}^2\text{ s}^{-1}$).

In all our measurements, the phospholipid recovery was $\sim 100\%$ whereas that for lipopolysaccharide was $\geq 75\%$. In each case, recovery was monophasic. Thus, there is no evidence of an immobilised 'boundary' lipid; on a time scale of seconds—the time required for diffusion over micrometre distances—all the lipid is exchangeable.

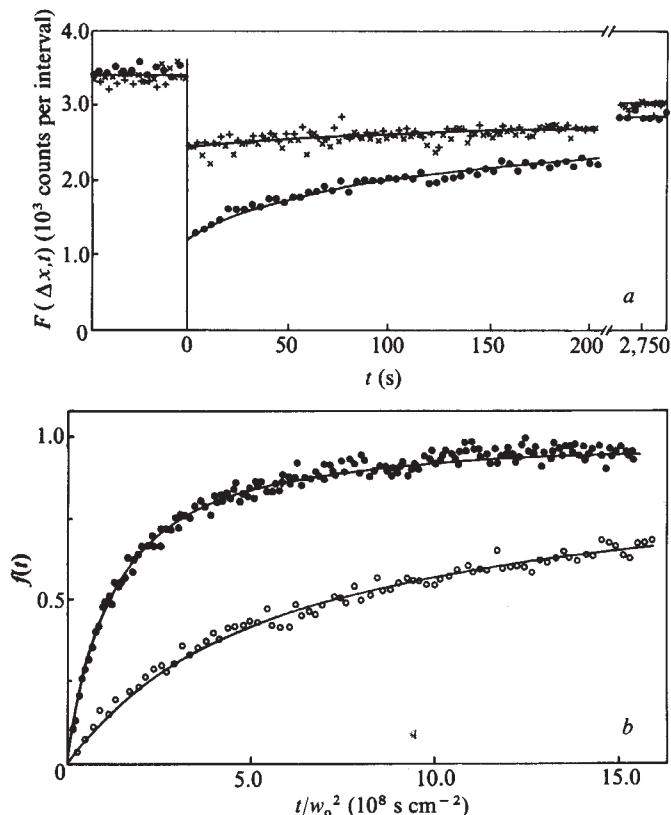


Fig. 4 *a*, Fluorescence recovery curves for rhodamine-labelled lipopolysaccharide in a multilayer membrane 1:1.3:1 by weight phospholipid/protein/lipopolysaccharide. The fluorescence was monitored coincident with the bleaching pulse, that is, $\Delta x = 0$ (●), and at equal distances on either side of centre, $\Delta x = \pm 3.0\ \mu\text{m}$ (+, ×). The solid curves are theoretical values corresponding to $\beta = 0.80$, $\tau_D = 153\text{ s}$, $w_0^2 = 10.8\ \mu\text{m}^2$, and thus, $D = 1.76 \times 10^{-10}\text{ cm}^2\text{ s}^{-1}$. Fluorescent components were derivatised in the following way. Electrodialysed G30 lipopolysaccharide (5 mg) was dispersed in 1 ml of 2% NaHCO_3 , pH 8.5. Rhodamine isothiocyanate (5 mg; Kodak) was added and the reaction allowed to proceed for 1 h at 4 °C. The reaction mixture was then chromatographed on a G-25 Sephadex column (1.5 × 25 cm) eluted with water. The rhodaminated lipopolysaccharide was eluted in the void volume, lyophilised, and then resuspended in 1 ml water. The suspension was washed three times with acetone. The lipopolysaccharide precipitated was judged pure by paper chromatography. NBD-PE (Avanti) demonstrated one spot on chromatography. Rhodamine modification of matrix protein involved the addition of 1 mg rhodamine isothiocyanate to a solution of matrix protein (2 mg ml^{-1}) in 2% SDS and 10 mM HEPES, pH 7.5. The reaction was allowed to proceed overnight at 4 °C. The protein was then precipitated five times in cold 90% acetone. The residue was lyophilised and then solubilised in 10 mM HEPES, pH 7.5. Amino acid analysis indicated that one amino group was modified per protein molecule. In addition, circular dichroism showed that the solubilised protein and rhodaminated protein maintained their predominantly β -sheet conformation (data not shown). Fluorescently labelled analogues of each component were incorporated at molar ratios between 1:100 and 1:1,000 to the unlabelled species. *b*, Fractional recoveries for NBD-PE and rhodamine-labelled lipopolysaccharide measured on the same area of a membrane 1:1.3:1 by weight phospholipid/protein/lipopolysaccharide. The theoretical curves correspond to a ratio of $D_{LPS}/D_{PL} = 0.14$.

Biomembranes as polymer networks for diffusion

The lateral mobility of cell membrane components is usually interpreted in terms of a fluid mosaic model²⁵. The membrane is pictured as a two-dimensional viscous solution with diffusion coefficients controlled by the membrane viscosity. The theory for diffusion in a two-dimensional fluid was put on a rigorous basis by Saffman and Delbrück²⁶. They showed that to a first approximation diffusion coefficients would be inversely proportional to the viscosity of the membrane and the vertical length of the diffusing molecule within the membrane. The theory demonstrated only a weak dependence on the cross-sectional area of the diffusing entity. In the context of the fluid mosaic model one would thus predict the following: (1) molecules as similar in lateral dimension as phospholipid (0.54 nm^2 cross-sectional area) and lipopolysaccharide (2.50 nm^2 cross-sectional area per monomer, molecular weight 3,600)²⁷ should diffuse at very nearly the same rate, and (2) differences in membrane composition or temperature should change diffusion coefficients by altering membrane viscosity, and hence should affect all coefficients proportionately. These predictions are obviously at odds with our experimental results for multilayers containing protein.

The data presented in Fig. 5 show that as the concentration of matrix protein increases in the membrane, the mobility of lipopolysaccharide is greatly reduced whereas that for phospholipid remains essentially constant. Moreover, as the temperature increases from 24 °C to 37 °C the diffusion constant of lipopolysaccharide increases significantly whereas that for phospholipid is unchanged.

Our results can be explained in a general way by reintroducing the idea that for some purposes, a biomembrane behaves like a polymeric network. The concept of biomembranes as polymers was first introduced by Lieb and Stein^{20,21} to explain transverse diffusion (non-mediated transport) of non-electrolytes across erythrocyte membranes. These studies were later extended to other membranes and demonstrated that, as with polymeric networks, (1) diffusion is a sensitive function of size, (2) diffusion is correlated with the short dimension of the diffusing particle, (3) as the temperature increases, the diffusion rates increase correspondingly more for larger particles than for smaller ones, (4) adding low molecular weight 'plasticisers' (for example, anaesthetics) increases the overall diffusion rates. The results strongly supported the hypothesis that transmembrane diffusion is more accurately described by comparison with non-porous networks of hydrophobic polymers than simple viscous liquids. We propose that the same analogy can be made for translational diffusion of membrane components within the plane of the membrane.

A simple physical model can help provide an understanding of the proposed polymer characteristics. The random thermal motion of polymer chains can be thought of as forming free volumes or 'holes' into which the diffusing molecules can move. The rate of diffusion would then be controlled by the rate of appearance of holes and the probability distribution of hole sizes. Net motion will occur only when a hole is created that exceeds a certain critical size approximately equal to the size of the diffusing molecule. This model thus predicts a sharp size-dependent rate of diffusion. Anything that decreases the average hole size will preferentially slow down the diffusion rate of larger diffusing molecules.

Specific interactions between matrix protein and lipopolysaccharide^{14,16,18} could also lead to differential diffusion changes. Such an association mechanism by itself, however, cannot account for the magnitude of the effect observed at low and intermediate protein concentrations. Theoretical values of D_{LPS} were calculated for a model in which specific binding occurs. It was assumed that bound lipopolysaccharide, which would be essentially immobile, is in rapid exchange with free lipopolysaccharide, consistent with our observations of a monophasic recovery. D for free lipopolysaccharide was

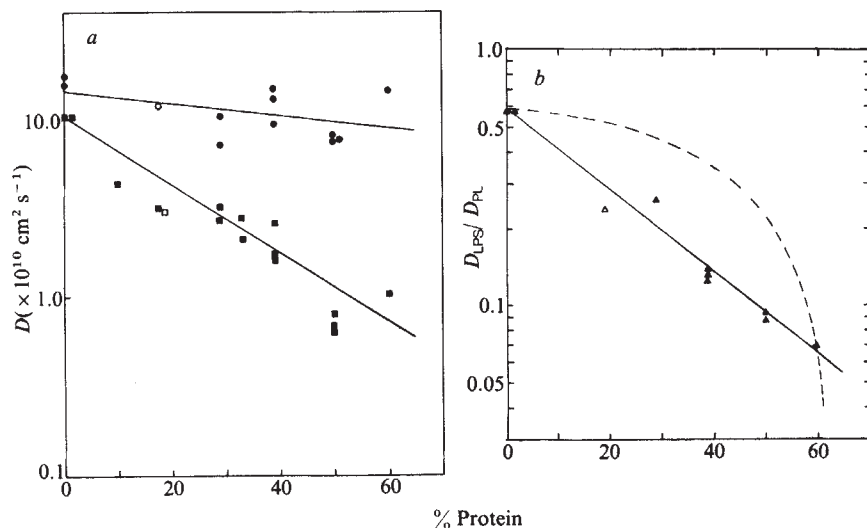


Fig. 5 *a*, Diffusion coefficients of NBD-PE (●, ○), and rhodamine-labelled lipopolysaccharide (■, □) as functions of the percent protein in multibilayer membranes. *b*, Ratios of D_{LPS}/D_{PL} . All ratios were determined from pairs of consecutive measurements carried out on the same patch of double-labelled membrane. The dashed line is a theoretical plot the significance of which is described in the text. The open symbols indicate a preparation with a 12:1 (w/w) ratio of phospholipid/lipopolysaccharide. All others correspond to a 1:1 ratio.

assumed to vary with protein concentration in a form identical to that found for phospholipids. The dashed curve in Fig. 5*b* corresponds to a stoichiometric binding, with an infinite association constant, of three lipopolysaccharide molecules to a matrix protein monomer¹⁸ in membranes with a phospholipid/lipopolysaccharide ratio of 1:1 by weight. Values calculated for lower association constants would deviate from the experimental curve even more significantly. In addition, these calculations predict that for a given protein concentration, D_{LPS} should decrease as the ratio of lipopolysaccharide to protein is decreased. In fact, however, at a protein concentration of 20%, D_{LPS} for a sample with a lipopolysaccharide to protein molar ratio of 3 (open symbols) was essentially indistinguishable from that of samples with a lipopolysaccharide to protein molar ratio of 20.

In the context of the fluid mosaic model, various attempts have been made to relate lateral diffusion to rotational mobility interpreted as membrane microviscosity^{1,28}. In our view of membranes as polymeric networks, there need be no simple relationship between lateral and rotational diffusion. Molecules free to rotate rapidly in limited regions might not be able to move laterally over large distances; and changes in membrane composition that moderately affect rotation rates could have profound effects on the lateral diffusion²⁸.

The concepts as outlined are particularly useful in relating our findings to the outer membrane of *E. coli*. Our multilayer results indicate that lipopolysaccharide and phospholipids are relatively mobile. However, other evidence suggests that the outer membrane is rigid, with largely immobile components^{29,30}. This difference can again be explained in terms of polymer domains. Underlying the outer membrane is a highly cross-linked peptidoglycan structure that is covalently anchored to the outer membrane³¹. The peptidoglycan could thus control the diffusion of outer membrane components. Depending on the degree of cross-linking or attachment of proteins, it is conceivable that some areas exist in the outer membrane that allow greater degrees of mobility than other areas. This might have particular relevance to growth zones in the membrane and phage infectivity.

Schlessinger *et al.*³² reported diffusion characteristics in cells in which the state of aggregation of one receptor affects the diffusion of another (anchorage modulation hypothesis). These results can be explained by changes in the polymeric nature of the membrane mediated by cytoskeleton components (microfilaments, microtubules).

Investigations of lateral mobilities of proteins in neonatal erythrocytes by Tokuyasu *et al.*³³ have shown membrane domains free of an underlying spectrin network. Lateral mobility in these domains was demonstrated to be significantly greater than in the spectrin-containing areas.

Because our data and most other mobility-related observations can successfully be interpreted using a polymer network approach, we believe that such an analysis might provide a general guide to membrane events. In addition, it would be able to reconcile the dual aspect of membranes—their viscoelastic properties, such as hyperelasticity and shear, and their fluid dynamics. The fluid mosaic model in our interpretation would be a special case of the polymer membrane in which the degree of polymerisation approaches zero.

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The role of gene deletion in the immunoglobulin heavy chain switch

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We have examined the arrangement of the genes for the mouse heavy chain subclasses γ_{2b} , γ_{2a} and γ_1 by the Southern hybridisation procedure. Evidence has been found for rearrangement involving the γ_{2b} and γ_{2a} C_H genes in the DNA of cells making IgG2b and IgG2a respectively. The DNA of an IgG2b-secreting line lacks detectable C_{γ_1} gene, whilst the DNA of an IgG2a-secreting line lacks detectable C_{γ_1} and $C_{\gamma_{2b}}$ genes. The DNA of a cell line secreting IgA lacks detectable C_{γ} genes. These observations implicate deletion in the mechanism of the H-chain switch and allow a preliminary ordering of some of the C_H genes in the mouse genome.

IMMUNOGLOBULIN polypeptides comprise two heavy (H) and two light (L) chains, each in turn consisting of an amino-terminal variable (V) region and a carboxy-terminal constant (C) region. The H-chain constant regions are sub-divided into five subclasses (μ , δ , γ , ϵ , α) each of which associates with a V-gene at a certain stage of B-cell differentiation. In 1965, the two genes—one polypeptide hypothesis¹ suggested that the V and C regions were separately encoded and that an integration event brought these two segments together. Extensive data now exist on the events involved in the differentiation of the L-chain genes in mouse. For example, it is now known that kappa (κ) chain genes have separately encoded V and C segments²⁻⁴ which are rearranged in the formation of the active κ gene^{2,3,5}. The C_{κ} gene, in germ-line DNA, is separated by intervening sequences from a set of segments called J-segments²⁻⁵. Integration involves fusion of a V_{κ} segment with one of the J segments and the intervening sequence, being retained between J and C (refs 2-7), is removed post-transcriptionally by RNA splicing enzymes^{8,9}. In the H-chain gene system the C_{μ} seems to be the initial expressed H-chain class^{10,11}. However, H-chain gene expression involves the H-chain switch that is manifested by the predominance of IgM (μ -chain class) in the primary response and by the predominance of IgG (γ -chain class) in the secondary response¹¹. Also the terminal stage of this pathway can be, in some cases, a switch to IgA (α -chain class) production¹¹. At the DNA level this switch would seem to involve a V_H gene first being expressed with one C_H gene (such as C_{μ}), then being 'switched' from this C_H gene to a different one (such as C_{γ}) and possibly later being switched to a further different C_H gene (such as C_{α}). A critical feature of the switch is the expression of a V-region idiotype with the different C_H classes^{12,13}. Several hypotheses have been proposed to account for the switch: principal among these are:

- (1) *Sequential insertion model.* An initial event integrates a V_H gene with the C_{μ} gene and subsequently this V_H gene is re-integrated with a C_{γ} gene and finally with C_{α} (ref. 14).
- (2) *Multiple insertion model.* The integration event involves integration of copies of a V_H gene with each C_H gene¹⁴.
- (3) *Post-transcriptional model.* A single integration event is invoked at the most distal part of the C_H gene's array. The V_H gene plus C_H gene array is transcribed as a unit into a precursor RNA which can yield V_H - C_H combinations by RNA splicing^{8,15}.
- (4) *Deletion model.* Integration occurs between a V_H gene and the C_{μ} gene with subsequent switching facilitated by deletion of material between the integrated V_H gene and the next C_H gene¹⁶.

Each of these theories is testable since the various C_H genes can be regarded as markers for H-chain gene arrangements and

rearrangements. Honjo and Kataoka¹⁶ have used cDNA probes (made by reverse-transcribing mRNA) to test these hypotheses by solution hybridisation and they concluded that the H-chain gene switch operates by a deletion mechanism. We have examined the hybridisation of cloned cDNA plasmids, containing γ_{2b} and γ_1 sequences, with genomic DNA made from cells producing different Ig H-chain classes. The results indicate rearrangement involving γ_{2b} and γ_{2a} genes in the DNA of cells producing the respective immunoglobulins and are consistent with the idea that the H-chain switch at least partly involves deletion events.

Cross-hybridisation of mouse γ subclasses

The cloned cDNA probe used in the experiments described here was $p\gamma_{2b}/7$, a cDNA complementary to part of the C_H region of γ_{2b} cloned in the plasmid vector pMB9 (this clone was prepared by Schibler, Marcu and Perry^{17,18}). This probe has been used in Southern hybridisation experiments¹⁹ after labelling with ³²P by nick-translation^{20,21}. Briefly, the hybridisation procedure involves agarose gel electrophoresis of restriction enzyme digested DNA followed by immobilisation of the DNA on cellulose nitrate filters. These filters were hybridised to the ³²P-labelled cloned DNA (or cDNA transcribed from purified mRNA with reverse transcriptase), washed and hybridisation bands visualised by autoradiography.

Cross-hybridisation has been reported between the γ subclasses in liquid hybridisation²². We have examined the cross-hybridisation between γ_{2a} , γ_{2b} and γ_1 gene sequences using the filter hybridisation method. The $p\gamma_{2b}/7$ clone is cleaved by the restriction enzyme *Hha*I to yield a fragment of about 2.2 kilobases which contains the γ_{2b} cDNA insert¹⁷. We found by screening a number of different restriction enzymes that the γ_{2b} cDNA insert was internally cleaved by the enzymes *Sac*I, *Pvu*II, *Pst*I, *Kpn*I, *Bst*I (unpublished data). Accordingly $p\gamma_{2b}/7$ DNA was digested with *Hha*I alone or *Hha*I plus one of these enzymes, fractionated on agarose gels and the DNA transferred to cellulose nitrate filters. These filters were hybridised with either nick-translated ³²P-labelled $p\gamma_{2b}/7$ DNA (Fig. 1a) with cDNA made from purified γ_{2a} H-chain mRNA (Fig. 1b) or with cDNA made from purified γ_{2b} H-chain mRNA (Fig. 1c). Clearly there is no distinction between the hybridisation of cDNA made from either γ_{2a} or γ_{2b} mRNA and the digests of the $p\gamma_{2b}/7$ clone. Both of these cDNA probes (Fig. 1b, c) hybridise specifically to the restriction fragments containing γ_{2b} sequence whilst the ³²P- $p\gamma_{2b}/7$ clone DNA hybridises in addition to plasmid-derived restriction fragments (Fig. 1a). The presence of cleavage sites for the various enzymes within the γ_{2b} insert is

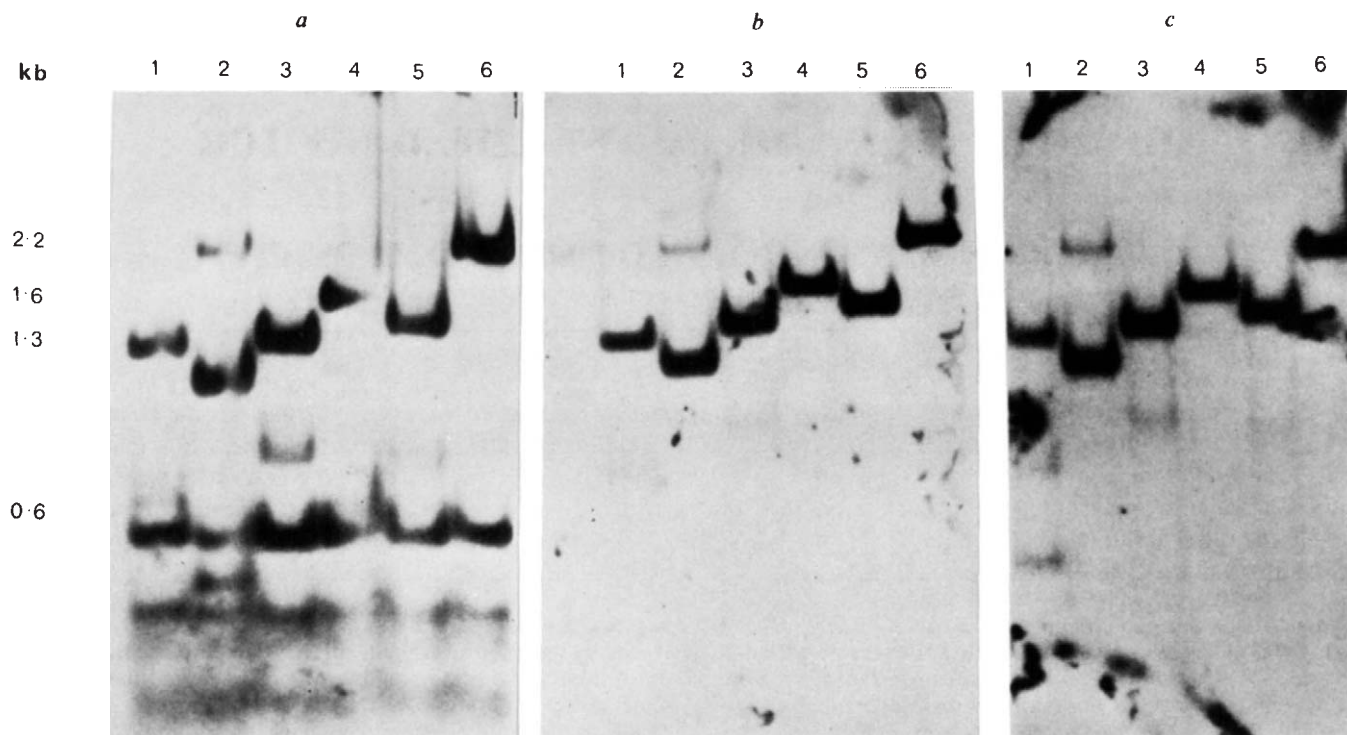


Fig. 1 Cross-hybridisation of $p\gamma_{2b}/7$ and cDNA made from cells producing γ_{2a} or γ_{2b} H-chain mRNA. 100 ng $p\gamma_{2b}/7$ were digested to completion with *HhaI* and further digested with *SacI*, *PvuII*, *PstI*, *KpnI* or *BstI*. The digests were fractionated by electrophoresis on 2% agarose gels, the DNA was denatured and transferred to cellulose nitrate filters¹⁹. The filters were subsequently hybridised to *a*, nick-translated ^{32}P -labelled $p\gamma_{2b}/7$ DNA; *b*, T_{10} -primed ^{32}P -labelled cDNA synthesised on γ_{2a} H-chain mRNA using reverse transcriptase²; or *c*, T_{10} -primed ^{32}P -labelled cDNA made on γ_{2b} H-chain mRNA. The hybridisations were carried out for 36 h at 65 °C in $6\times\text{SSC}$, 0.1% SDS, $50\text{ }\mu\text{g ml}^{-1}$ denatured sheared salmon DNA, $10\text{ }\mu\text{g ml}^{-1}$ poly(A) plus Ficoll, bovine serum albumin and polyvinyl pyrrolidone as prescribed by Denhardt⁴⁰ and Jeffreys and Flavell⁴¹. Post hybridisation washes were carried out at 65 °C, first with $6\times\text{SSC}$ containing the above components and then five washes (30 min in each) with $1\times\text{SSC}$ plus the above additions. A final wash with $1\times\text{SSC}$, 0.1% SDS was carried out before overnight autoradiography with prefogged X-ray film at -70 °C (ref. 42). Slot 1, *HhaI*+*SacI*; slot 2, *HhaI*+*PvuII*; slot 3, *HhaI*+*PstI*; slot 4, *HhaI*+*KpnI*; slot 5, *HhaI*+*BstI*; slot 6, *HhaI* alone. Molecular weights are given in kilobases (kb).

manifested by reduction in the size of *HhaI* fragments after redigestion with the appropriate enzyme. Evidently, therefore, γ_{2a} and γ_{2b} sequences cross-hybridise with more or less equal efficiency in the hybridisation conditions used. A similar approach has been used to demonstrate the cross-hybridisation of γ_1 and γ_{2b} sequences (data not shown).

Hybridisation analysis of the genes for the γ subclasses in various myeloma cell lines

The various cell lines or tumours used in this study are listed in Table 1 and each cell line was monitored for the production of the respective type of immunoglobulin (by *in vitro* incorporation of ^{14}C -labelled amino acids). The strategy of our experiments has been to use the genomic DNA of these cell lines to hybridise (using the Southern filter hybridisation procedure) with probes for γ_2 and γ_1 genes using hybridisation conditions established in the previous section, where γ_2 and γ_1 genes cross-react to varying degrees. Figure 2 shows the result of a Southern hybridisation between the cloned γ_{2b} DNA and *EcoRI*-digested DNA of various types. The kidney DNA (slot 4) shows two major hybridisation components of about 20 and 7 kilobases respectively. Kataoka *et al.*²³ have recently isolated a genomic DNA clone (from *EcoRI*-digested mouse DNA) which was shown to contain the γ_{2b} C_H-gene by partial nucleotide sequencing. This cloned DNA has a size of around 7 kilobases and allows an unequivocal assignment of the 7-kilobase component as containing the γ_{2b} gene. Interestingly, we find that DNA from cells synthesising γ_{2a} showed only the large

hybridising component (slot 2). Since the 20-kilobase fragment is the only hybridisation component observed in this DNA, it can be assigned to the fragment containing the γ_{2a} C_H-gene and the similar component of the kidney DNA will therefore represent the γ_{2a} gene. The hybridisation of γ_{2b} DNA to *EcoRI*-digested DNA of IgG2b-producing cells (Fig. 2, slot 3) and IgG1-producing cells (slot 1) show essentially the same pattern as kidney DNA, except that the IgG1 DNA exhibits a new hybridisation band (about 4.3 kilobases): the origin of this component is not yet known. The data in Fig. 2 show that we cannot detect the γ_{2b} C_H-gene in cells producing IgG2a although we were able to detect this gene and the γ_{2a} C_H-gene in the other DNAs examined.

Two main possibilities may explain our inability to detect the γ_{2b} gene in the IgG2a-producer DNA. Either the gene is absent from this DNA or the H-chain switch involves rearrangements of the γ_{2b} C_H-gene in this DNA in such a way that γ_{2a} and γ_{2b} genes appear in a single fragment. The latter possibility is ruled out by the data in Fig. 3 where we have compared the hybridisation of γ_{2b} cloned DNA to either liver DNA or IgG2a-producer DNA digested with various enzymes. *BstI* or *BstI* plus *EcoRI* digests of liver DNA yielded hybridisation bands of 6.7, 5.5 and 5.0 kilobases (Fig. 3), whereas the IgG2a-producer DNA only yielded a 5.5-kilobase fragment. This latter fragment must, therefore, represent the γ_{2a} C_H-gene whilst the 5.0-kilobase hybridisation fragment seen in the liver DNA contains the γ_{2b} C_H-gene²³. The IgG2a-producer DNA appears not to contain this 5.0-kilobase γ_{2b} fragment. In addition, if the γ_{2b} gene were present in IgG2a-producer cells, *BstI* digestion (which cleaves

within the γ_{2b} coding region, see Fig. 1) should yield an additional hybridising fragment due to cleavage within the γ_{2b} sequence. As no such band appears we conclude that the 5.5-kilobase band does not include the γ_{2b} C_H-gene. This conclusion is corroborated by the results of *Kpn* digestion (Fig. 3). *Kpn* or *Kpn* plus *Eco*RI digestion of liver DNA yielded major hybridisation components at 18.5 and 5.1 kilobases whilst the IgG2a-producer DNA yielded only the 18.5-kilobase fragment. By arguments analogous to those used previously, the 18.5-kilobase fragment must represent the γ_{2a} C_H-gene and again, since *Kpn* cleaves within the γ_{2b} insert (Fig. 1), this

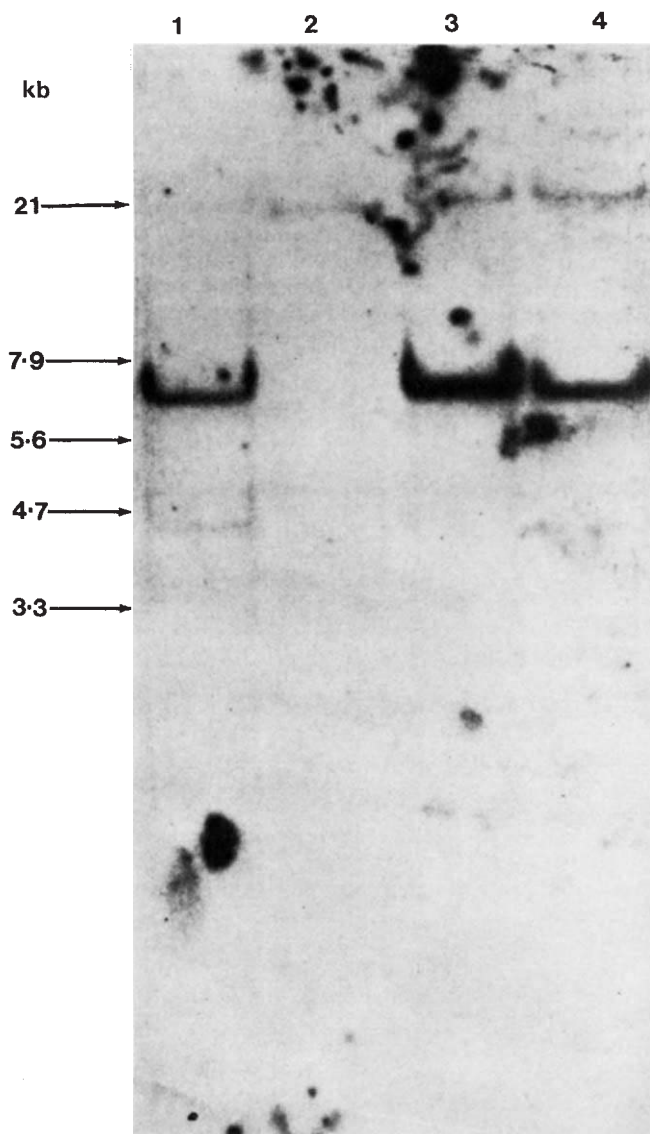


Fig. 2 Pattern of hybridisation of γ_{2b} cDNA to *Eco*RI-digested nuclear DNA from cells synthesising various C γ subclasses. High molecular weight nuclear DNA² from cells producing Ig of the classes γ_1 , γ_{2a} , γ_{2b} or DNA of kidney cells were digested to completion with *Eco*RI, fractionated by 0.8% agarose gel electrophoresis and the DNA transferred to cellulose nitrate filters¹⁹. Hybridisation of the filters was carried out for 48 h with 25 ng ml⁻¹ nick-translated p γ_{2b} /7 (specific activity 5×10^7 c.p.m. μ g⁻¹) in $6 \times$ SSC (hybridisation, post-hybridisation washing and autoradiography was carried out as legend to Fig. 1). This autoradiograph shows hybridisation to DNA of cells making γ_1 (slot 1), γ_{2a} (slot 2), γ_{2b} (slot 3) and DNA of kidney cells (slot 4). Size calibration was carried out by co-electrophoresis of an *Eco*RI digest of λ phage DNA followed by ethidium bromide staining before transfer of DNA to the filters.

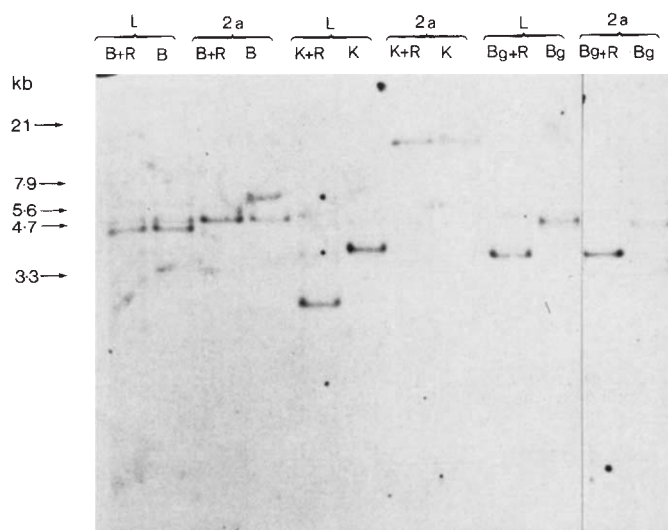


Fig. 3 Hybridisation of γ_{2b} cDNA to liver DNA and DNA of an IgG2a-secreting cell. Liver DNA or DNA prepared from an IgG2a-secreting cell were digested with the restriction enzymes *Bst*I, *Kpn*I or *Bgl*II or mixtures of these enzymes with *Eco*RI. The digests were fractionated and hybridised to ³²P-labelled p γ_{2b} /7 as in Fig. 2 legend. B = *Bst*I; B + R = double digest of *Bst*I + *Eco*RI; K = *Kpn*I; K + R = double digest of *Kpn*I + *Eco*RI; Bg = *Bgl*II; Bg + R = double digest of *Bgl*II + *Eco*RI; L = liver DNA; 2a = DNA of IgG2a-secreting cell.

18.5-kilobase fragment cannot contain the γ_{2b} gene as an additional hybridising fragment would appear in such a circumstance. The 5.1-kilobase *Kpn* fragment of liver DNA can be assigned to the γ_1 C_H-gene²⁴ so we conclude that we cannot detect a γ_1 C_H-gene in IgG2a-producer DNA. (This conclusion has been confirmed by the inability of a γ_1 cDNA probe to hybridise this C_H-gene in the IgG2a-producer DNA [unpublished data].) Furthermore, liver DNA yielded a strongly hybridising *Kpn* fragment of 4.2 kilobases which is cleaved by *Eco*RI: the intensity of this hybridisation suggests that the fragment contains the γ_{2b} C_H-gene, and this conclusion has been confirmed by restriction mapping of a genomic γ_{2b} C_H-gene clone²³. We do not observe any such component in the IgG2a-producer DNA which extends our conclusion that this DNA lacks the γ_{2b} C_H-gene.

The comparison of liver DNA and IgG2a DNA in Fig. 3 also includes the results of digestion with *Bgl*II which provides evidence for rearrangement events involving the γ_{2a} C_H-genes. The liver DNA cleaved by *Bgl*II produced two main bands of hybridisation of about 5.2 and 4.9 kilobases. Both of these bands are susceptible to *Eco*RI since double digests produce a new major band at about 3.9 kilobases. The IgG2a cell DNA shows only a single major hybridisation band of about 3.8 kilobases when digested with *Bgl*II plus *Eco*RI: *Bgl*II digestion alone, however, produced a distinctly different pattern of hybridisation compared to liver DNA. Now only the 4.9-kilobase fragment is common between liver DNA and the IgG2a cell DNA whilst new bands of about 8 and 6.3 kilobases occur in the IgG2a cell DNA. One or both of these bands presumably reflects rearrangements resulting from V_H-gene integration with the γ_{2a} C_H-gene within the DNA of cells destined to synthesise IgG2a. In summary, the data in Figs 2 and 3 support the argument that the DNA of the cell line producing IgG2a lacks C_H genes for γ_{2b} and γ_1 and shows that evidence of rearrangement events specifically involving the γ_{2a} C_H-gene can be found in cells making IgG2a.

Further analyses regarding the presence of the γ_1 and γ_2 genes in cells making IgM, IgG1 and IgA are shown in Figs 4 and 5.

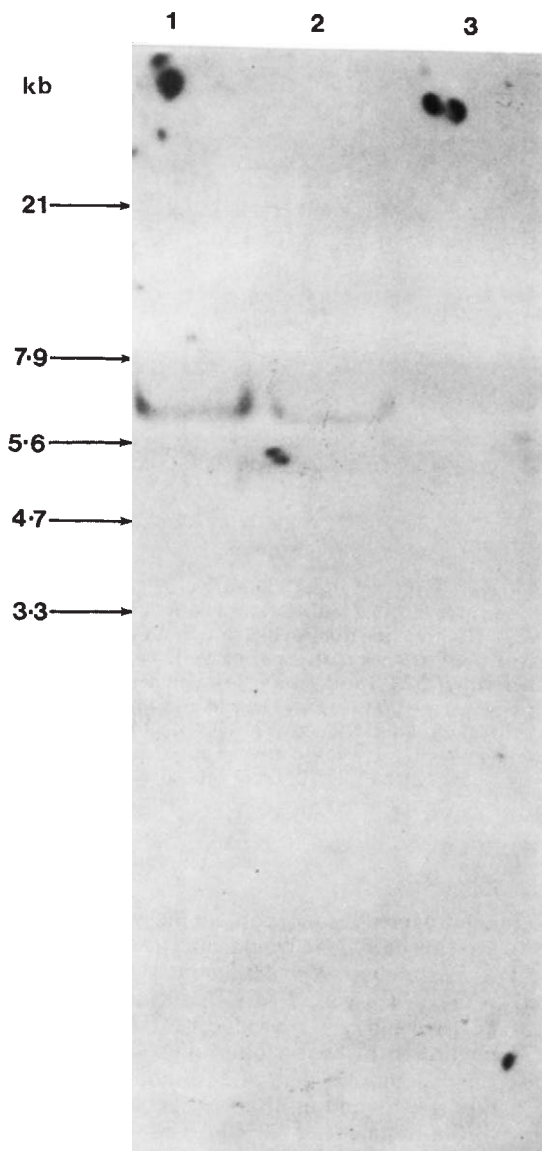


Fig. 4 Hybridisation pattern of γ_{2b} cDNA to DNA from cells making γ_{2b} or α heavy chains. As for Fig. 2, *EcoRI*-digested DNA of liver cells, of cells making IgG2b or IgA was electrophoresed, transferred to filters and these filters hybridised to ^{32}P -labelled $p\gamma_{2b}/7$ in $6\times\text{SSC}$ followed by washing and autoradiography. Samples loaded were DNA from γ_{2b} -producing cells (slot 1), from liver cells (slot 2) and from α -producing cells (slot 3).

Figure 4 shows the hybridisation patterns of $p\gamma_{2b}/7$ with *EcoRI* digests of DNA from the IgG2b secretor (slot 1), liver (slot 2) and an IgA secretor (slot 3). The DNAs of the IgG2b secretor and liver display the characteristic *EcoRI* hybridisation fragments of the γ_{2b} (about 7 kilobases) and the γ_{2a} C_H -genes (about 20 kilobases). The IgA cell DNA (Fig. 4, slot 3), however, failed to hybridise the $p\gamma_{2b}/7$ probe, indicating the absence of γ_{2b} and γ_{2a} genes from these cells. Although we do not have a cDNA probe for α -chains, these cells were shown to secrete IgA by *in vitro* incorporation of ^{14}C -labelled amino acids (data not shown). In addition, the same *EcoRI*-digested IgA cell DNA was found to hybridise normally to a κ -L-chain probe. We conclude, therefore, that this IgA-producing cell line does not possess γ_{2a} or γ_{2b} genes.

The presence of both γ_{2a} and γ_{2b} hybridisation components in the DNA of cell lines producing IgM or IgG1 plus clear evidence of rearrangement involving γ_{2b} C_H -genes specifically in the

DNA of cells making IgG2b is shown in Fig. 5. In Fig. 5, tracks 6–8 show hybridisation patterns of the $p\gamma_{2b}/7$ to various *EcoRI* digests. Track 6 contained *EcoRI*-digested DNA of the IgG2a secretor showing the 20-kilobase $C\gamma_{2a}$ hybridisation band. DNA of both the IgM secretor (track 7) and the IgG1 secretor (track 8) shows this hybridisation band. These DNA types also show the γ_{2b} C_H -gene hybridisation band of around 7 kilobases, showing that these cells possess the genes for γ_{2b} and γ_{2a} . Tracks 1–5 show hybridisation of the γ_{2b} probe to *KpnI*-digested DNA. The hybridisation of liver DNA and the IgG2a-producer DNA parallels that shown in Fig. 3 with the γ_{2b} C_H -gene fragment (4.2 kilobases) and γ_{2a} C_H -gene fragment (18.5 kilobases) occurring in liver DNA but only the latter in IgG2a cell DNA. The patterns of hybridisation to *KpnI* digests of IgG1 cell DNA (slot 1) and IgM cell DNA (slot 4) are identical to liver DNA, confirming that the γ_{2a} and γ_{2b} C_H -genes are present in these cells. The hybridisation of $p\gamma_{2b}/7$ to *KpnI*-digested DNA of the IgG2b secretor, however, shows a more complex pattern (slot 2). In addition to hybridisation of the γ_{2a} C_H -gene (18.5-kilobase component) and to the γ_{2b} C_H -gene (4.2 kilobases) we observed two new hybridisation bands of about 13 and 6.5 kilobases. These latter bands do not appear in any of the other DNA types studied and must, therefore, originate from rearrangement events which involved integration of the γ_{2b} C_H -gene in precursor cells of these IgG2b producers. These data also suggest that at least two chromosomes carrying γ_{2b} C_H -genes are present in these cells since we observe a mixed pattern

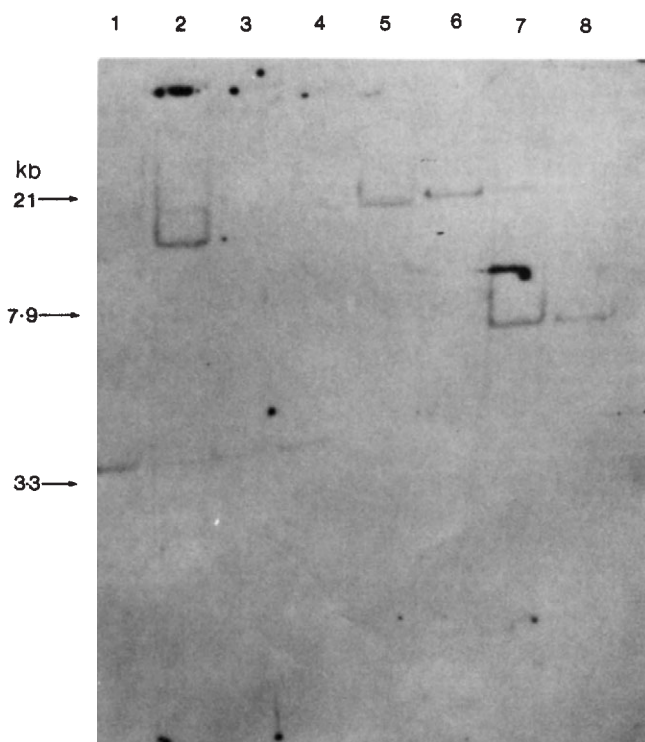


Fig. 5 Hybridisation pattern of γ_{2b} cDNA to *KpnI* and *EcoRI* digests of various mouse DNA. The DNA of liver cells or cells making heavy chains of the class γ_1 , γ_{2b} , γ_{2a} and μ were completely digested with *KpnI* and DNA of cells making γ_1 , μ and γ_{2a} were completely digested with *EcoRI*. The resulting DNA fragments were separated by 0.8% agarose gel electrophoresis, transferred to cellulose nitrate filters and hybridised and washed to ^{32}P -labelled $p\gamma_{2b}/7$ DNA in $1\times\text{SSC}$ conditions (in which γ_1 and γ_{2b} cross-hybridisation is minimised, unpublished observation). The autoradiograph shows the hybridisation patterns of DNA digested with *KpnI* (slots 1–5) and *EcoRI* (slots 6–8) and the DNA loadings in the slots are from cells of the type γ_1 (1), γ_{2b} (2), liver (3), μ (4), γ_{2a} (5), γ_{2a} (6), μ (7) and γ_1 (8).

Table 1 Cells used for hybridisation studies

Cell type	Mouse strain	Immunoglobulin produced
McPc 1748	BALB/c	IgM
MOPC 21	BALB/c	IgG1
P1	BALB/c	IgG2a
MPC 11	BALB/c	IgG2b
MOPC 315	BALB/c	IgA
Liver	BALB/c	—
Kidney	BALB/c	—

of the 'rearranged' γ_{2b} components plus a pattern similar to that seen in liver DNA.

The hybridisation conditions used in the type of experiment shown in Fig. 5 minimised the cross-hybridisation of γ_1 and γ_{2b} (unpublished observation). We have also tested for the presence of the γ_1 C_H-gene *Kpn* fragment in various DNAs in low stringency conditions where γ_1 and γ_2 do cross-hybridise (Fig. 6). Liver DNA and an IgM-producer DNA show (amongst other cross-hybridising genes) the 5.1-kilobase *Kpn* fragment characteristic of the γ_1 C_H-gene²⁴. The *Kpn*-digested DNA from the γ_{2b} -producer, however, fails to display this γ_1 C_H-gene component, indicating the absence of this gene from this DNA.

Restriction mapping data implicate gene deletion in the H-chain switch

The data presented in this article show that the DNAs of myeloma cells producing various immunoglobulins can be mapped with respect to their subclass genes using cloned cDNA sequences. This analysis revealed two major features. Evidence of rearrangement events involving C_H-genes for γ_{2a} and γ_{2b} was found in the DNA of cells expressing γ_{2a} or γ_{2b} genes respectively. These rearrangements, analogous to those observed by restriction mapping κ -chain genes^{2,3,5} presumably represent alterations of the DNA located around these genes during integration with the V_H gene in the creation of the active transcription unit. The alterations in this respect seem to be independent since we only find evidence of rearrangements specifically involving a C_H gene in the DNA of cells producing the respective class of H-chain, for example, evidence of γ_{2b} rearrangement could only be found in the DNA of cells making IgG2b and not, say, in cells making IgM. However, the alterations seem to interrelate to the extent that when γ_{2a} cell DNA is examined we find evidence of rearrangements affecting γ_{2a} C_H-genes in these cells, but no evidence of the continued existence of γ_{2b} genes in the DNA. Furthermore, the γ_{2a} and γ_{2b} cells lack detectable γ_1 C_H-genes, whilst μ and γ_1 -producing cells possess all three subclass genes. IgA-secreting cells fail to show evidence of any of these γ -subclass genes in their DNA. The presence or absence of the detectable genes in the tumours examined are summarised in Table 2 which includes gene assignments based on appropriate synthesis of Ig chains by cell cultures. Honjo and Kataoka have previously reported similar findings concerning the absence of various C_H genes in myeloma cell lines¹⁶. Previous evidence^{10,11} suggests that the switch goes from C μ to C γ to C α . Therefore cells making IgM must necessarily contain the C μ gene, the C γ subclasses, plus C α , whilst cells making IgA do not necessarily require the previously utilised C_H genes (such as C γ). In view of the pattern of absence of the C_H genes from the various tumours, therefore, the most compelling conclusion is that the absence of detectable C_H genes is the consequence of deletion operating during the switch of the V_H gene as previously proposed¹⁶. The switch operates intrachromosomally^{25,26} and the putative deletion event must therefore operate in the same way. It is known that there is close

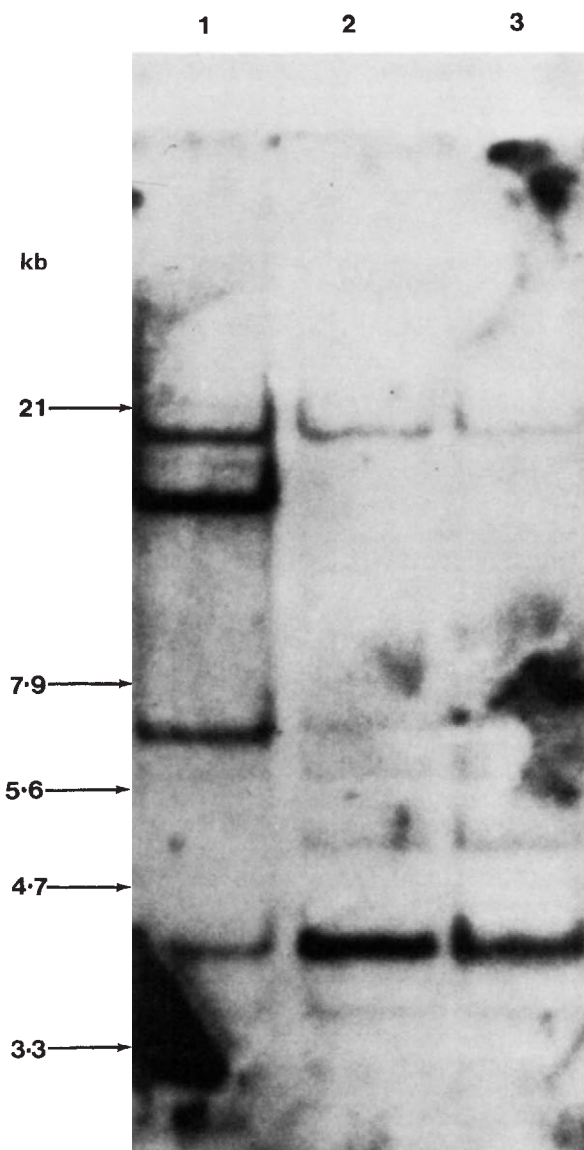


Fig. 6 Hybridisation pattern of *Kpn*I-digested nuclear DNA with γ_{2b} cDNA under low stringency hybridisation conditions. *Kpn*I-digested DNA from γ_{2b} -producing cells, liver cells or μ -producing cells was fractionated and hybridised as in Fig. 4 except that the lower stringency $6 \times$ SSC conditions were used. The slots shown are (1) DNA of γ_{2b} cells, (2) liver DNA, (3) DNA of μ cells.

linkage between the C_H genes in mouse²⁷. In a deletion process, the pattern of absence of C_H genes from cells would allow a preliminary spatial ordering of some C_H genes in the germ-line DNA. This ordering of C_H genes would appear to be C μ , C γ_1 , C γ_{2b} , C γ_{2a} , C α (Table 2) and an identical order of C_H genes was obtained by Honjo and Kataoka¹⁶ using solution hybridisation kinetics.

It is of interest to discuss the possible molecular basis of a deletion process particularly with reference to possible J_H segments. Integration of the V_L with C_L genes involves J segments which are fused with the V segment^{6,7,28-30}. It seems likely that similar J segments will occur in the H-chain system³¹ and that V_H-J_H fusion will be the initial step of integration of the V_H gene. Therefore we can expect that the selected V_H segment will fuse with one of an array of J_H segments, located close to the C μ gene. An intervening sequence probably separates the J_H and C μ of the newly created transcription unit (indeed it has been shown that an intervening sequence does occur between V and

Table 2 Summary of detection of H-chain genes showing probable arrangement of C_H genes in germ-line DNA

C _H gene expressed	Genes present and arrangement of C _H genes					
	μ	γ_1	γ_{2b}	γ_{2a}	α	
μ	✓	✓	✓	✓		ND
γ_1	ND	✓	✓	✓		ND
γ_{2b}	ND	×	✓	✓		ND
γ_{2a}	ND	×	×	✓		ND
α	ND	×	×	×		ND

✓, Relevant gene present in the DNA. ×, Relevant gene was undetected in the DNA. ND, not determined, as no probe was available.

C α in a clone isolated from cells making IgA³²), and the transcription unit now utilised by the cell will be V_{JH}... intervening sequence...C μ . Assuming that V_H-J_H fusion occurs similarly to the V_L-C_L events, the resulting gene segment will consist of a continuous base sequence of V_H and J_H. This sequence will not contain any non-coding bases between V and J which might act as signals for re-integration of the V_H to a new J_H segment in the switch. The switch must therefore operate differently from V_H integration. Two main possibilities exist. First, it might occur by unequal cross-over between sister chromatids before cell division. The site of recombination might be between similar or identical J_H segments associated with the C_H genes, or sequences occurring within the intervening sequence between the VJ-C μ transcription unit and between the various C_H genes could be used for the recombination event. The second possibility draws an analogy from the mechanisms proposed for κ -chain integration which invoke the pairing of repeat sequences before V κ -J κ fusion^{5,6}. Thus it is possible that the switch of the V gene involves such repeat sequences present both within the intervening sequence of the VJ-C μ transcription unit and within the sequences separating the various C_H genes. Pairing of such repeat sequences followed by excision of the looped-out material could then facilitate the switching of a V_H gene between C_H genes.

A deletion mechanism for the switch has previously been proposed on the basis of solution hybridisation data by Honjo and Katoaka¹⁶. These workers deduced that the switch operated on only one of the allelic chromosomes with the non-expressed chromosomes remaining unchanged. Our data suggest the different possibility that the switch occurs on both allelic chromosomes since the experiments show, apparently, the absence of C_H genes from both chromosomes. However, the aneuploid nature of myeloma karyotypes may indicate that the cells used have lost the non-expressed allelic chromosome. We must also consider the possibilities that the deletions which we have observed are merely the manifestation of the aberrant myeloma karyotypes and as such would have no particular bearing on the H-chain switch. However, the observation that rearrangements involving the C_H genes of γ_{2a} and γ_{2b} can be specifically detected in the respective secretor DNA together with the apparent absence of certain C_H genes in the same DNA argues for the authenticity of the conclusion. In addition, experiments testing the rearrangement of some κ -producing myeloma cells^{1,2} also indicated possible rearrangement of the non-expressed allelic chromosome, so chromosome loss may not be the full explanation. We are left with the possibility that the V/C integration (both L and H chain) and even the H-chain switch occurs on both allelic chromosomes, and it is possible that such alterations on both chromosomes may explain the presence of extra hybridisation bands in certain digests of γ_{2a} and γ_{2b} DNA.

The involvement of deletion in the H-chain switch may not be the whole answer to the problem, since cells clearly cannot go back to express a gene which has been deleted. Therefore we can

ask the question about the situation regarding cells which co-produce μ and δ -H-chains^{33,34}. A possible explanation is that these cells contain stable mRNA from one or other of these genes so that continued production of the protein can occur after switch/deletion to the second gene. However, it is still possible that μ - δ co-production occurs by V-C joining in the nuclear RNA precursors. Such a general mechanism was proposed for the H-chain switch^{8,15,35} but it has been shown that at least μ and γ C-gene sequences do not co-exist in the nuclear RNA precursors¹⁸. However, the nuclear RNA precursors of μ / δ -producing cells may contain a precursor of the type V_HC μ C δ from which can be made either mRNA V_HC μ or V_HC δ by RNA splicing. This would be a simplification of previous schemes^{8,15}. In the context of possible RNA splicing or recombination it is interesting that variant myeloma cells have been detected which show evidence of switching within the γ subclasses³⁶⁻³⁹. These cells may or may not be representations of the normal switch events and DNA cloning of the variant genes should settle this problem.

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LETTERS

Radio emission from radio-quiet quasars

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In a search for radio emission from bright, optically selected quasars, we detected ~50% of the quasars at 5 GHz. This radio detection rate is so much higher than that for faint quasars (<10%)¹⁻⁴, that it requires that the distribution of radio(R)-to-optical(O) luminosity ratios (L_R/L_O) be correlated with optical properties and/or with distance (or cosmic epoch). The values of L_R/L_O in our sample range from $\sim 10^{-2}$ to $<10^{-5}$ (where radio detection is no longer possible). So broad and smooth a distribution argues against a distinct dichotomy between 'radio-quiet' and 'radio-loud' quasars. The quasars in our sample which remain undetected at radio frequencies must exhibit a spectral-flux distribution rising from the radio to optical, previously considered an 'abnormal' spectrum for a quasar.

Although quasars were originally discovered on the basis of strong radio emission, it now seems that most optically selected quasars are undetectable as radio sources¹⁻⁴. In fact, most optically selected quasars show no clear evidence, at any frequency, for synchrotron radiation. For most quasars, whether radio-quiet or radio-loud, the IR-optical emission is not highly polarised ($\leq 1\%$) (refs 5, 6), not a simple power law⁷, and not highly variable⁸. In contrast, the radio emission from virtually every extragalactic radio source, and the IR-optical continuum radiation in a small subset of quasars, namely, the BL-Lac objects^{9,10} and the 'optically-violent-variable' (OVV) quasars¹¹, seems explicable in terms of the synchrotron process.

There is still no obvious way to predict, on the basis of IR-optical properties, that a given quasar is radio-quiet⁷. Indeed, how quiet is 'quiet'? If there were a distinct dichotomy between 'radio-quiet' and 'radio-loud' quasars¹², the distribution of radio-to-optical luminosity ratios (L_R/L_O) would probably be bimodal. On the other hand, if radio-quiet and radio-loud quasars are merely extrema of the same phenomenon (as is the case for models involving geometric orientation, such as the relativistic-jet model¹³⁻¹⁵), the distribution of (L_R/L_O) for optically selected quasars should be very broad and smooth.

To answer the question, 'How quiet is quiet?', and to examine the distribution of radio-to-optical luminosity ratios (L_R/L_O), we initiated a search for radio emission from bright, optically selected quasars, with the very large array (VLA) of the US National Radio Astronomy Observatory (NRAO). Using the 1.5-m Mt Lemmon (Arizona) telescope of the University of Minnesota (UMinn) and University of California at San Diego (UCSD), we also obtained optical and IR photometry to seek correlations between IR-optical properties and radio emission.

The investigation reported here differs in two respects from most previous searches for radio emission from optically selected quasars¹⁻⁴. First, we considered only the optically brighter, optically selected quasars—typically, at least 10 times brighter than the optical limits used in previous searches. Second, we used the VLA at 4.885 GHz: At this frequency, the VLA is roughly 10 times more sensitive than any other radio telescope. Combining these two factors then, the present investigation probes the radio-to-optical luminosity-ratio (L_R/L_O) distribution down to values ~100 times smaller than those

examined in previous searches. In fact, the limiting spectral flux of the 5-GHz search is even slightly smaller than the intended optical limit of the sample selected—namely, 0.6 mJy at 703 THz ($B = 17$ mag). Consequently, to escape radio detection, a quasar from this optically selected sample must have an inverted spectrum—that is, its spectral-flux distribution (perhaps due to the superposition of multiple components) must rise from radio to optical frequencies.

We now briefly describe the samples and the results. A more detailed presentation, including the IR and optical photometry and possible interpretations in terms of physical mechanisms, appears elsewhere¹⁶.

The sample consists of 22 bright, optically selected quasars (Table 1), studied previously¹⁷⁻²¹ and contained in the quasar catalogue of Burbidge, Crowne and Smith²². During our radio and IR-optical observations (spring 1978 to spring 1979), only 16 of the selected quasars had a B magnitude brighter than 17. While the sample is not complete, it is, we believe, unbiased with respect to radio emission: quasars are neither included nor excluded because of their radio properties.

We detected 9 of the 22 quasars, including 8 of the 16 with $B \leq 17$, at a level >0.6 mJy at 4.885 GHz. Most of the radio sources are unresolved on the VLA, and thus are more compact than a few arc seconds. The detection rate is much higher than that of any previous search for radio emission from optically selected quasars; nevertheless, about half the quasars in our bright-quasar sample remain undetected at radio wavelengths, with a standard deviation of 0.15 mJy about a mean of 0.06 mJy. Therefore, as Fig. 1 shows, more than half the quasars have spectral-flux distributions increasing from radio to optical frequencies, that is, negative radio-to-optical spectral indices, $\alpha_R^O < 0$, for $\alpha_R^O \equiv -[\Delta(\ln F_\nu)/\Delta(\ln \nu)]$.

The distribution of α_R^O exhibits no clear separation between 'radio-loud' and 'radio-quiet': it appears smooth from $\alpha_R^O \approx 0.7$ down to $\alpha_R^O \approx 0.0$, at which point the radio emission is no longer detectable for every quasar in the sample. Thus we find no evidence to support the conjecture that 'radio-loud' and 'radio-quiet' quasars belong to two distinctly different populations¹².

Elsewhere¹⁶, we consider in detail plausible explanations for the observed large range in radio-to-optical luminosity ratios. If

Table 1 Effective radio-to-optical spectral indices of bright, optically selected quasars

Coordinate designation	Redshift	Spectral flux at 702 THz (mJy)	Radio-to-optical spectral index	Refs Sample Photometry
0007+106	0.089	5.63	+0.36	21 16
0024+224	1.118	0.68	+0.48	19 16
0026+129	0.142	2.33	-0.02	21 16
0054+144	0.171	1.68	<-0.12	20 16
0100+130	2.690	0.58	<-0.03	20 16
0205+024	0.155	2.11	-0.03	19 16
0847+190	0.568	0.56	<-0.03	20 16
0848+163	1.932	0.8	<-0.06	20 22
0906+484	0.118	0.75	<-0.05	21 16
1001+054	0.161	0.97	<-0.07	21 16
1011+250	1.631	0.90	+0.55	17 16
1246+377	1.241	0.47	<-0.01	18 8
1257+346	1.375	0.54	+0.27	18 16
1304+346	0.184	0.82	<-0.06	18 16
1317+277	1.022	1.39	<-0.10	17 16
1318+290	0.549	0.69	<-0.05	17 16
1321+294	0.960	0.67	<-0.04	17 16
1351+640	0.088	4.41	+0.16	21 7
1517+239	1.901	0.34	<+0.01	20 16
1523+214	1.924	0.21	<+0.05	20 16
1525+227	0.253	0.75	+0.43	20 16
2344+092	0.677	1.09	+0.63	21 16

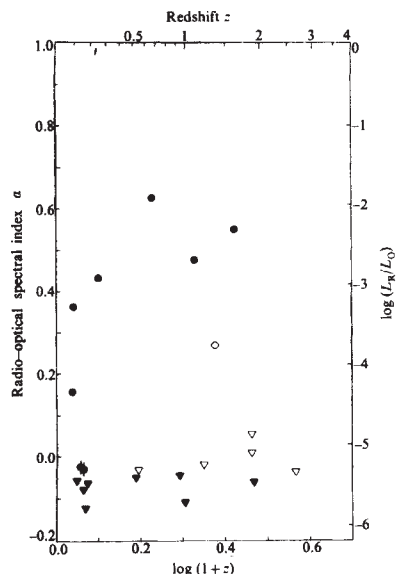


Fig. 1 Distribution of radio-to-optical luminosity ratios (L_R/L_O) for a sample of bright optically selected quasars, plotted against $(1+z)$, with z the redshift. $\circ$, Radio detections; ∇ , 2σ upper limits. The filled symbols represent the brighter quasars ($B < 17$ mag).

the IR-optical emission from quasars is synchrotron (or processed, for example, Compton scattered, synchrotron) radiation, the frequent weakness of synchrotron emission at radio frequencies might result from synchrotron self-absorption or from free-free absorption by intervening material, such as broad-line emitting regions. (This latter explanation is quite reasonable; however, it would be inadequate if truly radio-quiet BL-Lac objects were to be discovered.) If the IR-optical continuum is not synchrotron radiation, there need not be any correlation between radio and optical luminosities. Relativistic-jet models^{13,14} owing to differences in orientation, require a rather broad distribution of radio-to-optical spectral indices^{15,23}. In this respect, our results are in qualitative agreement with relativistic-jet models; however, agreement in detail requires an hypothesis somewhat more complicated than that considered so far¹⁵ (see below).

Finally, we question why the radio-detection rate for our sample ($\sim 50\%$) is so much higher than that for other searches ($\sim 10\%$)¹⁻⁴. That we consider only optically bright quasars and have utilised the remarkable sensitivity of the VLA partly explains the higher detection rate for our sample. However, even with a 100-fold less sensitive radio search ($F_r < 60$ mJy, or 100 mJy, say), we would still have detected about 30% (5 out of 16) of the quasars with $B \leq 17$ mag.

It seems, therefore, that quasars of bright apparent magnitude exhibit a significantly higher incidence of radio emission than would be expected on the basis of a scaling of detection thresholds. In as much as the optical-selection techniques are unbiased with respect to radio emission, the fraction of quasars with (L_R/L_O) or $(-\alpha_R)$ above some threshold should be independent of the limiting optical flux or any other optical-selection criterion provided that the ratio (L_R/L_O) is uncorrelated both with optical properties and with distance (and, hence, cosmic epoch). That this fraction does depend on the limiting optical flux, shows that the distribution of (L_R/L_O) must depend on optical properties and/or epoch (or distance). Owing to the small size of the present sample, one cannot readily determine which of these dependences is more important (see Fig. 1); however, the more rapid evolution observed for optically selected quasars, in comparison to that observed for flat-spectrum radio sources²⁴, suggests that evolution in the distribution of (L_R/L_O) plays some part.

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A new mode of X-ray bursts from MXB1730-335

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We report here on the unusual behaviour of MXB1730-335, the rapid burster¹ as observed by the X-ray astronomy satellite Hakucho between 8 and 22 August 1979². MXB1730-335 is a unique burst source which generates rapidly repetitive bursts lasting between a few and tens of seconds. In the present observations, however, its behaviour was very different to that previously observed, in that the bursts of a trapezoidal profile were dominant. The profile is characterised by a flat top lasting between 30 s and 10 min. A long train of such trapezoidal bursts was seen for the first time and clearly revealed a new mode of the rapid burster.

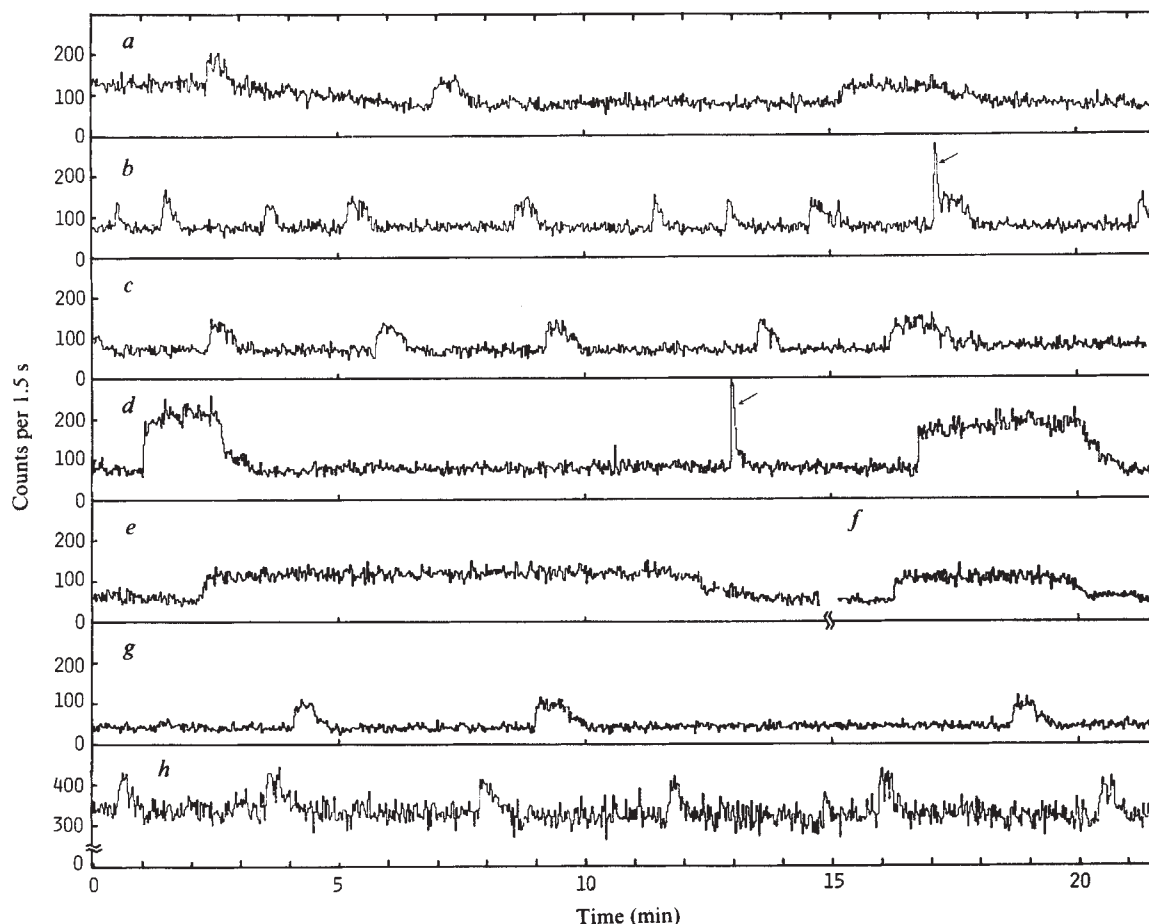


Fig. 1 Segments of the X-ray count-rate record. The graphs *a*–*g* are from counter FMC 2, and *h* is the sum of the rate from CMC 1 and 2. Since the exposure of the target and the covered energy range (*a*, *b*, 1.5–10 keV; *d*–*h*, 3–10 keV) are not corrected for, displayed count-rates should not be directly discussed. Two bursts indicated by arrows are from MXB1728–337, a nearby burster. Each segment starts at the following times (UT): *a*, 8/8, 1 h 55 min 19.2 s; *b*, 8/8, 3 h 37 min 39.8 s; *c*, 8/8, 5 h 19 min 45.3 s; *d*, 8/8, 19 h 48 min 00 s; *e*, 8/11, 19 h 19 min 54.3 s; *f*, 8/12, 16 h 08 min 24.1 s; *g*, 8/14, 17 h 22 m 58.3 s and *h*, 8/17, 13 h 43 min 39.1 s.

The observation was carried out with an X-ray burst monitor which consists of two sets of rotating modulation collimators: two coarse modulation collimators (CMC 1,2) and a fine modulation collimator (FMC 1) with a field of view of 17.4° and 5.8° (FWHM), respectively. Positions of steady sources and the burst, when it occurs, can be determined with the burst monitor to an accuracy of 0.5° or better. An additional counter (FMC 2) with 5.8° field of view (FWHM) provides non-modulated source signal for comparison. Further details of the instrumentation and the performance of Hakucho are described elsewhere³.

Since 31 July the burst monitor has been observing a sky region which includes the galactic centre. At 0 h 10 min UT, 8 August, a source in the field of view, whose position was determined with the CMC in a 0.5° error circle including MXB1730–335, began to generate a train of bursts (the source was soon confirmed by the FMC 1). The activity of the source was watched through 22 August. The turn-on of the rapid burster was roughly on schedule as expected from the suggested periodicity^{4,5} of ~6 months: the previous active period was early March 1979⁶.

Figure 1 shows several segments of the X-ray count-rate record. In the early period of the activity starting at 0 h 10 min UT of 8 August, unusual bursts of a trapezoidal profile appeared among bursts of a normal exponential shape (Fig. 1*a*). The data from 3 h 37 min to 4 h 00 min UT of 8 August (Fig. 1*b*) show characteristic features of bursts from the rapid burster such as those observed by SAS 3 (refs 1, 7). After a pause of data acquisition for ~6 h, a long burst train entirely trapezoidal in

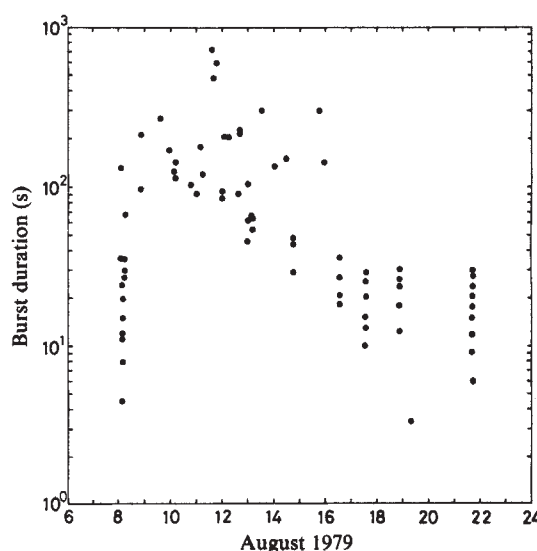


Fig. 2 Plot of the burst duration measured at half maximum of the burst height. Not all the bursts, particularly those of short duration, are plotted. Most bursts of the duration longer than 30 s are of trapezoidal shape. The error of the duration is ~3 s and ~5 s for the period before and after 17 August respectively.

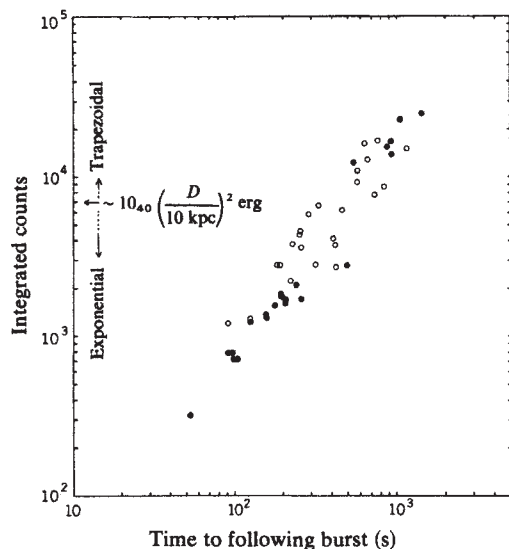


Fig. 3 Integrated counts for a burst over 1.5–10 keV versus the time to the following burst. Correspondence between the total count within an error of $\pm 15\%$ and the total energy is indicated on the ordinate. Note, however, that the energy estimate is tentative. ●, 8.0–11.8 August; ○, 12.5–18.9 August 1979.

form appeared (Fig. 1d, e). About 90 trapezoidal bursts were observed between 8 and 16 August during a total exposure time of ~ 35 h.

The predominance of such trapezoidal bursts is new property of the rapid burster⁷, although a few such unusual bursts from the rapid burster were noted during the previous turn-on period (ref. 6 and personal communication from W. Lewin).

The burst durations (FWHM) observed for selected satellite orbits are plotted in Fig. 2. The burst duration increased soon after the turn-on of the rapid burster, exceeding 10 min at its maximum. After 12 August trapezoidal bursts of shorter duration predominated (Fig. 1g), while the duration scatters. Then, on 16 August bursts of an exponential shape again appeared among trapezoidal bursts and after 17 August, the feature became similar to that of the normal rapid burster (Fig. 1h). The activity continued until 22 August when we lost MXB1730–335 from our sight because of the solar direction constraint.

The time profile of the trapezoidal burst is characterised by the following features: a fast rise within ~ 1 s, a nearly constant plateau and a slow fall to half the maximum in 10–30 s with the association of a shoulder or an after pulse in the decaying phase. These features seem to be independent of the pulse width. The peak luminosity of the trapezoidal bursts is of the order of $10^{38} (D/10\text{kpc})^2 \text{ erg s}^{-1}$, D being the distance to the source, but is found to vary by as much as a factor of four. From a simple analysis, the spectral hardness ratio is found to remain constant within statistical uncertainty throughout the flat portion of the trapezoidal burst.

For cases when two or more bursts occurred in an uninterrupted observation window, the integrated counts in each burst (burst size) are plotted against the time interval to the succeeding burst in Fig. 3. While the estimate is tentative, the total energy for most of the trapezoidal bursts $> 10^{40} (D/10\text{kpc})^2 \text{ erg}$. The correlation between the burst size and the interval is similar to that shown by the SAS 3 result (ref. 7 and refs therein); that is, the larger the burst size, the longer is the time until the next burst.

The average slope of the points in Fig. 3 is somewhat steeper than a linear relationship. Whereas, we note episodes in which the burst size versus interval relation is closely linear and hence the time-averaged luminosity is about constant. For example, the filled circles in Fig. 3 indicate two such episodes. The slope is greater than unity because an increased average luminosity tends to favour a larger burst, although the burst size fluctuates.

The average luminosity was found to vary by a factor of three over the period of the present observation, during which the burst size spanned two orders of magnitude.

The train of trapezoidal burst is probably a new mode of the Type II burst (ref. 7 and refs therein) which is presumably caused by an instability of the accretion flow onto the neutron star surface. The present data indicate that the trapezoidal bursts predominate when the average luminosity is above a certain value. This implies that a relatively small change in the mean accretion rate corresponds to a drastic change in the burst profile. Instead of increasing the frequency of usual bursts of exponential shape, trapezoidal bursts of various widths are produced with the peak luminosity near the Eddington limit for a $1 M_{\odot}$ star. Note, however, that this peak luminosity is also variable. This newly discovered mode of burst undoubtedly imposes a strong constraint on the model of the rapid burster.

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Neutral ^{14}N in the interstellar medium

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The transition frequencies between the hyperfine structure sublevels of atomic nitrogen lie in the decimetre waveband. As Shklovsky¹ predicted, these transitions can be observed in the form of narrow spectral lines. The ^{14}N ground state ($^4\text{S}_{3/2}$, nucleus spin $I = 1$) is split into three sublevels $F = 5/2, 3/2$ and $1/2$ with frequencies and transition probabilities

$$\nu = 26.127 \text{ MHz}, \quad A = 4.32 \times 10^{-20} \text{ s}^{-1} \text{ for } F = 5/2 \rightarrow 3/2$$

and

$$\nu = 15.676 \text{ MHz}, \quad A = 1.29 \times 10^{-20} \text{ s}^{-1} \text{ for } F = 3/2 \rightarrow 1/2$$

The Einstein coefficients given here have been calculated from the improved values of line intensities². We describe here an attempt made between 1978 and 1979 at the Institute of Radiophysics and Electronics, Academy of Science of the Ukrainian SSR, to detect the ^{14}N absorption line for some point and extended cosmic objects. This effort has been successful for Cassiopeia A.

The observations were carried out using the UTR-2 radio telescope³ at a frequency of $26.13 \pm 20 \text{ kHz}$ (collective area of the antenna about $5 \times 10^4 \text{ m}^2$). The emission spectrum was measured with a spectrometer⁴ consisting of a receiver with a frequency synthesiser, a digital correlator and a computer controlling the antenna beam and converting the autocorrelation function into the spectrum. Measurements of autocorrelation functions were taken with the beam directed to the source of interest alternatively by the maximum and the first zero (for between 0.5 and 1 s). The two values were further subtracted instrumentally. The difference autocorrelation functions were stored and converted into the spectrum. The total storage time of the difference autocorrelation functions was from 1 to 5 h in one observation night. The resolution varied in different observation sessions from 1 to 4 kHz.

The 26.13 MHz absorption line was first detected in the signal of Cas A on 17 June, 1978 after which measurements carried

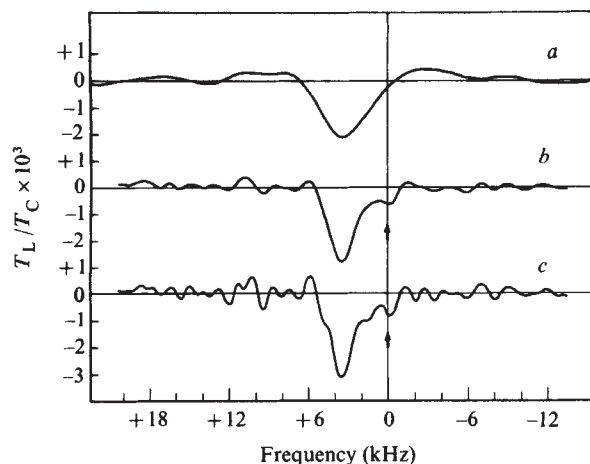


Fig. 1 Absorption spectra in the ^{14}N line $F=5/2-3/2$ for Cassiopeia A. a, Resolution 4 kHz, storage time $T=26$ h; b, resolution 2 kHz, $T=25$ h; c, resolution 1 kHz, $T=24$ h. The arrow shows the frequency corresponding to the Orion arm.

out over the next year established a coincidence of the observed changes in the mean frequency of the line with the orbital Doppler shift (0.67 kHz with respect to the standard of rest).

Figure 1 shows three spectra from Cas A plotted with different resolutions in the frequency- T_L/T_C scale (where T_L and T_C are temperatures in the line and the continuum). Figure 2 shows the predicted Doppler frequency shift due to the orbital motion of the Earth and measured mean frequencies of the line for different observations. Figure 3 shows the H I absorption line in terms of velocities instead of frequencies⁵ and the ^{14}N line reduced to a zero orbital velocity. Strong absorption can be seen in the Perseus arm ($V = -38$ to -48 km s $^{-1}$) and weak absorption in the Orion arm ($V = -0.8$ km s $^{-1}$). The measured optical depths at the line minimum are 3×10^{-3} for the Perseus arm and 5×10^{-4} for the Orion arm. The expected absorption line width in the Orion arm⁵ is ~ 0.4 kHz. Hence, the intensity of this part has been underestimated in our measurements.

Thus, the periodic change of frequency coinciding with the orbital Doppler effect and comparing the H I in Cas A spectrum suggests that we have discovered the $F=5/2 \rightarrow 3/2$ line of ^{14}N .

Measurements for Cygnus A have shown that in its direction $\tau < 2 \times 10^{-4}$; measurements for the Galaxy centre were greatly hampered by interference from broadcasting stations and only allowed us to estimate $\tau < 6 \times 10^{-4}$.

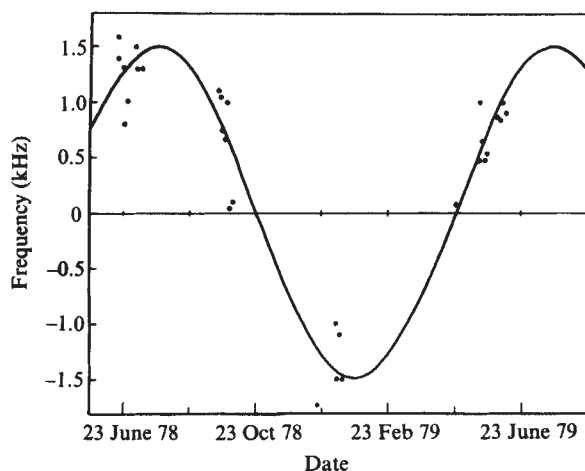


Fig. 2 Change of frequency due to the orbital motion of the Earth. Solid line, calculations; ●, measurements.

To estimate the abundance of nitrogen in the absorbing arms we use the relationship

$$S = \int \tau(\nu) d\nu = \tau_{\max} \Delta\nu_{\text{eff}} = \frac{\hbar c^2}{4kT_s} \frac{A}{\nu} \frac{g_F}{\sum g_F} n$$

where $\tau(\nu)$ is the line profile; $\hbar$, k , c are Planck's constant, Boltzmann's constant and the velocity of light, respectively; A , and ν are the probability and frequency of the transition; g_F the statistical weight of the sublevel; T_s the spin temperature; and n the density of atoms.

For ^{14}N we have $n_N = 0.7 \times 10^{18} S_N T_{SN}$ (subscript N denotes the values for nitrogen). Our measurements give $S_N \approx 6$ s $^{-1}$ for the Perseus arm, hence assuming $T_{SN} = 50$ K, we find $n_N = 2.8 \times 10^{20}$ cm $^{-2}$.

Comparing the abundance of ^{14}N using the H I line, in accordance with Schutter and Verschuur⁵, S_H can be estimated as $S_H \approx 10^6$ s $^{-1}$, and with $A_H = 2.85 \times 10^{-15}$ s $^{-1}$ and $\nu_H = 1.42 \times 10^9$ Hz we obtain

$$\frac{n_H}{n_N} \approx 130 \frac{T_{SH}}{T_{SN}}$$

If we assume $T_{SH} = T_{SN}$, the abundance of nitrogen with respect to hydrogen would be more than one order of magnitude higher than the average value in the Galaxy (that is 5×10^{-4}). Other explanations of the unexpectedly strong absorption in the ^{14}N line are also possible.

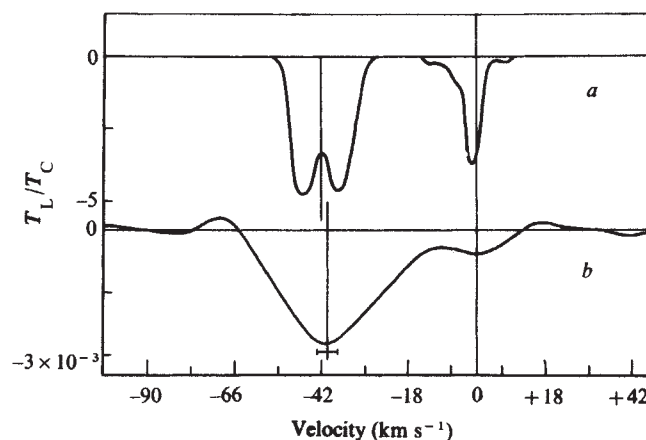


Fig. 3 Velocity spectra in the absorption clouds of the Orion and Perseus arms. a, H I line, 1,420 MHz; b, ^{14}N line, 26.13 MHz.

(1) The true value of S_H in the gas cloud of the Perseus arm is $S_H \gg 10^6$ s $^{-1}$. Other measurements⁵ allow any value of $\tau_H > 4.7$. In this case the estimate for hydrogen density would need to be increased by more than an order of magnitude and the OH/H I relationship (see ref. 6) revised assuming a higher value for τ_H .

(2) The spin temperature $T_{SN} \ll T_{SH}$: this could be the result of either some specific features in the population of the ^{14}N levels (which can be checked by a theoretical calculation) or nitrogen distribution in the colder parts of the gas cloud.

Further investigations are planned with the aim of discovering the line $F=3/2 \rightarrow 1/2$ ($\nu = 15.7$ MHz).

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Catalytic combustion in a tube with electrical discharge: preliminary measurements of augmented reaction rates

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Since Senftleben¹ first indicated that in the presence of electric fields, heat transfer rates may be increased substantially beyond natural convection values, correlations have been proposed relating electric field and fluid flow parameters to film coefficients of transfer. The 'corona wind', which exists in a non-uniform electric field before spark breakdown, can be used to alter the hydrodynamics of convective diffusion processes such that an augmentation of transport rates in wall boundary layers can be achieved²⁻⁶. Corona wind augmentation of wall reactions, particularly catalytic combustion processes, has not previously been considered^{7,8}, but as catalytic combustion is a convective diffusion process, the superposition of the corona wind on the flow field near the catalytic surface may augment overall reaction rates by increased mixing. For very rapid surface reactions, where molecular diffusion inhibits the overall rate and lowers overall rate constants, corona wind augmentation may limit the effects of diffusion and, thereby, increase catalyst effectiveness. Improvements in the overall rate constant, or mass transfer coefficient, would be useful in many areas, including air pollution control, energy conservation, and the design of compact, controlled heat flux catalytic combustors. We report here our test of the catalytic combustion of hydrogen at one atmosphere on platinised alumina in a flow reactor with a uniformly accessible catalyst surface. By this experimental design we may be able to obtain reaction rate data from the kinetically controlled to the diffusion limited regimes of combustion owing to the high activity of hydrogen on metallic platinum. A uniformly accessible catalyst surface also permits analysis of the reaction rate data using either one- or two-dimensional approaches.

The experimental reactor is an annulus of large radius ratio, and the corona discharge is applied from a small diameter (0.053 cm) stainless steel wire suspended under tension along the centreline. The outer cylinder is a 95.5% Al_2O_3 tube of dimensions 4.50 cm o.d. $\times$ 3.91 cm i.d. $\times$ 58.4 cm long. This tube is coated internally with platinum at an average of 13.3 g m^{-2} . The reactor wall is maintained at a constant temperature to within $\pm 5^\circ\text{C}$ with electrical heaters, and bulk flow temperatures are held at about one-half the wall value to minimise the possibility of gas phase reaction. The catalyst surface is grounded and a positive d.c. potential (300 V maximum a.c. ripple over the range 6–11 kV) is applied to the central wire. Premixed hydrogen and air enter the reactor in nearly fully developed flow. The combustion of hydrogen is determined by measurements of both inlet and outlet bulk concentrations with a gas chromatograph.

Reaction rate data as a function of average mole per cent of hydrogen, $\bar{y}$, at a constant corona current, I , at a wall temperature, T_w , of 427°C are presented in Fig. 1. At this wall temperature, the reaction is diffusionally limited in laminar flow⁹⁻¹⁰, and the rate data exhibit the expected first-order dependence on $\bar{y}$. Rate data at lower wall temperatures¹¹ are also first order with respect to $\bar{y}$. From the data of Fig. 1, increases in the reaction rate of the order of 20–25% are observed for moderate levels of corona current. These increases may be interpreted as an augmentation of the mass transfer coefficient owing to the uniformly accessible catalyst surface¹².

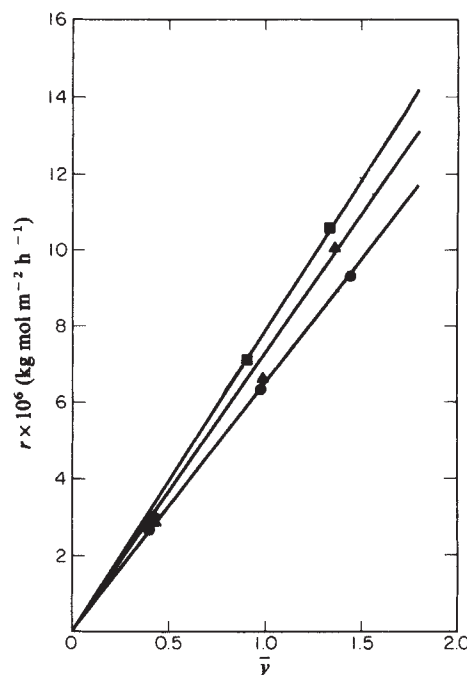


Fig. 1 Reaction rate for combustion of hydrogen on platinised alumina at constant corona current where $T_w = 427^\circ\text{C}$; $T_B = 260^\circ\text{C}$; $Re = 1,437$. $\blacksquare$, $I = 500 \mu\text{A}$; $\blacktriangle$, $I = 300 \mu\text{A}$; $\bullet$, $I = 0$.

The combined roles of the Reynolds number and corona current are shown in Fig. 2. Reaction rate data versus corona current for given values of Reynolds number and inlet concentrations of hydrogen, y_i , are presented in Fig. 2a in terms of the fractional increase of reaction rate due to the corona discharge. The range of corona current is somewhat larger than shown in Fig. 1 and includes all of the values possible before spark breakdown. For a given value of I , the increase in reaction rate depends on the Reynolds number and y_i in a complex fashion (Fig. 2b). At low Reynolds number, the reaction is nearly complete, and $\Delta y/y_i$ is close to unity. Thus, the presence of the corona discharge results in a small increase in reaction rate even though corona wind velocities are of the order of the bulk convective velocity. At larger Reynolds numbers, $\Delta y/y_i$ is smaller, but because the bulk convective velocity is large enough to eliminate essentially the influence of the corona wind, a reasonable augmentation of the rate is obtained. At still larger Reynolds numbers, for example, $Re = 2,617$ in Fig. 2a, greater fractional increases in reaction rate are obtained. At this Reynolds number, the flow is probably turbulent, but apparently not of large enough velocity to wipe out the influence of corona discharge on the hydrodynamics at the catalytic wall. Limitations of the experimental reactor prohibited us from obtaining reaction rate data at larger Reynolds numbers at this wall temperature. However, one can expect that at larger Reynolds numbers, the axial convective transport would completely dominate the mass transfer problem and that the corona discharge would result in no augmentation of the reaction rate. A similar transition to turbulent flow behaviour has been observed in the heat transfer problem for both tube flows and external boundary layers¹³⁻¹⁴. Note that a cross-plot of data similar to that of Fig. 1 would yield different fractional augmentation values, but the same conclusions would be reached on the role of the Reynolds number.

When the reaction rate data are plotted as a function of Reynolds number at a constant corona current, the fractional augmentation of reaction rate exhibits the highly non-linear behaviour shown in Fig. 2b. Here, increases in Reynolds number past a certain value (depending roughly on wall temperature and corona current) result in a rapid decrease in $(r_e - r_0)/r_0$. This is

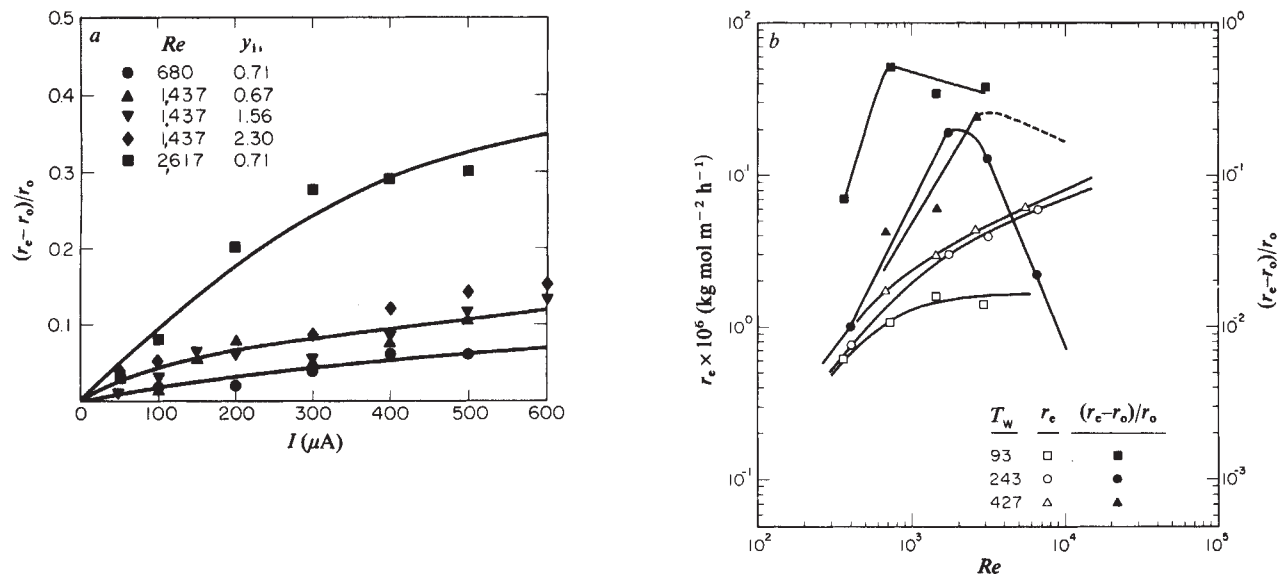


Fig. 2 a, Fractional increase in reaction rate as a function of corona discharge current: $T_w = 427^\circ\text{C}$; $T_B = 263^\circ\text{C}$. b, Reaction rate and augmented reaction rate as functions of Reynolds number at constant corona current ($I = 300 \mu\text{A}$).

particularly evident at $T_w = 243^\circ\text{C}$, where the augmented reaction rate rapidly approaches the value for no corona current as the Reynolds number attain values normally associated with turbulent tube flows. Note also that fractional augmentation of reaction rate at a given Reynolds number exhibits a complex dependence on wall temperature. This is shown in Fig. 2b where $(r_e - r_0)/r_0$ for $T_w = 93^\circ\text{C}$ is much larger than at $T_w = 243^\circ\text{C}$ and 427°C even though the overall reaction rate is lower. Additional experiments are needed, however, to determine completely the dependence of augmented reaction rates on I , Re and T_w .

The degree to which molecular diffusion limits the overall reaction rate is expressed in terms of the effective first-order rate constant derived from a one-dimensional analysis of the overall reaction rate data^{9,10}. Rate constants without corona discharge present exhibit a transition from mixed kinetics at $T_w = 93^\circ\text{C}$ to nearly diffusion controlled at $T_w = 427^\circ\text{C}$ (Fig. 3a). The presence of corona discharge produces the increase in rate constants as shown in Fig. 3b for a narrow range of Reynolds numbers. Here it is seen that, at $T_w = 427^\circ\text{C}$ where rate

constants can be interpreted as mass transfer coefficients^{9,10,12}, the corona discharge can produce up to a 50% augmentation in the rate constant. The error bars on each of the data points in Fig. 3b represent the maximum variation in the measured values.

Analysis of these experimental data has revealed no general mass transfer correlation for the data at $T_w = 243^\circ\text{C}$ and 427°C . Tentatively, we conclude that the augmentation of the reaction rate, or the rate constant, at these temperatures varies as $I^{1/2}$ at a constant Reynolds number. This result is reassuring in view of the work of Velkoff and Godfrey¹³ and Mizushima *et al.*¹⁴. However, additional experiments and analysis are needed to permit a mass transfer correlation of general engineering utility. For the data in the regime of mixed kinetics, that is, $T_w = 93^\circ\text{C}$, it is doubtful that diffusional and kinetic effects can be separated for a wide range of corona current.

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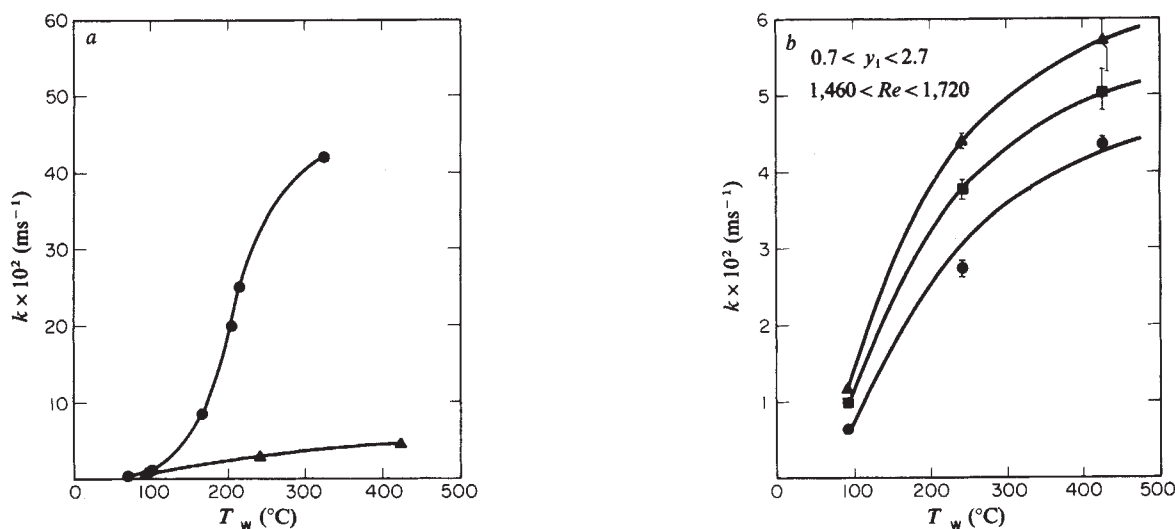


Fig. 3 a, Comparison of overall first-order rate constants of the present study (▲) with true rate constants (●) measured by Kulacki and Gidaspo. b, Overall first-order rate constants determined from a one-dimensional analysis of the reaction rate data. Curves are matched by eye. ▲, $I = 500 \mu\text{A}$; ■, $I = 300 \mu\text{A}$; ●, $I = 0$.

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Seasonal change in the flux of organic carbon to the deep Sargasso Sea

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Recent evidence^{1–5} has suggested that diurnal and annual periodicities affect the deep sea which traditionally had been thought to be the least variable environment at the surface of the Earth^{6,7}. We report here what we believe to be the first direct measurements of seasonal change in the total flux of particles <13 cm and in the flux of organic carbon associated with those particles. The change seems to be related to seasonal change in the primary productivity of the surface water.

Between April 1978 and July 1979 a series of samples of particulate matter were collected in a sediment trap similar to the type described by Honjo⁸. The trap has a collecting cross-section of 1.54 m² and could accept particles <13 cm. The largest particles collected, in fact, were about 10 mm in length. Collection periods ranged from 60 to 68 days. The trap was moored 1,000 m above the sea floor and intercepted the particle flux at a depth of 3,200 ± 100 m. It did not collect during its descent or its ascent. The collection site was 25 nautical miles south-east of Bermuda. The experiment was not designed primarily to measure the organic carbon flux. No preservative or poison was contained in the collection cup of the sediment trap, but their absence is not expected to cause significant loss of organic carbon at that depth within 2-month collection periods⁸.

Data on the total particle catch, on the mass distribution of particles smaller and greater than 125 µm, and on organic carbon content are shown in Fig. 1. The data are averaged over the collection periods. The last two sampling periods cover approximately the same time of year as the first two. For comparison, Fig. 1 also shows the monthly averages of the daily net primary productivity measured by Menzel and Ryther⁹ between December 1957 and April 1960. Those measurements were made at a location 10 miles from the mooring site of our sediment trap.

Figure 1 shows: (1) fluctuation of a factor 2.75 in the amount of particulate matter collected per unit time; (2) relatively smooth variations of the amounts through the 16 months, with highs in April/May and lows in the summer months; (3) similar variations in the size fractions and the organic carbon as in the total amounts; (4) an indication of some year-to-year variation,

with the 1979 high about 1.7 times as high as the 1978 one; and (5) similar locations of the highs and lows in the particulate flux and in the primary productivity.

Most of the material collected in the trap was of biogenic origin, with clay and other non-biogenic mineral particles representing only a minor part of the <37-µm size fraction. Most of the material consisted of whole individuals or fragments of Pteropoda, Foraminifera, Radiolaria, Gastropoda, diatoms and coccoliths, with occasional otoliths, squid beaks, fragments of bryozoan colonies and plant debris. Faecal pellets in various states of preservation were common and may have been more so in the undisturbed suspension in the collection cup of the trap. The organic carbon content was determined only on subsamples of the <125-µm fraction because of the greater heterogeneity of the coarser material and the destructive nature of the measurement. However, the <125-µm fraction comprised 82 ± 4% of the total samples and we do not believe that extrapolation of the organic carbon content as measured on that fraction over the entire samples introduces a significant error. The organic carbon in all samples had a rather uniform $\delta^{13}\text{C}$ of $-21.4 \pm 0.3\%$ relative to the PDB standard, a typical value of open-ocean particulate organic carbon¹⁰.

The data suggest that the flux to the deep sea of particles and of organic carbon in all but very large particles not captured by the trap is not uniform with time. There is indication of a seasonal cycle in that supply which, in the positions of its maximum and minimum, is similar to the seasonal cycle in primary production measured by Menzel and Ryther⁹. Unless the food supply to the deep-sea benthos consists predominantly of the very large particles not collected by a sediment trap of this type, these data represent the first indication of fluctuations in the supply of organic matter to the deep sea remote from major land areas. Seasonal variations in the standing crop of organic aggregates had been noted earlier by Riley *et al.*¹¹. The highs and lows in the supply are apparently linked to the highs and lows of primary production in the surface waters and thereby point towards a possible link between seasonality of surface productivity and seasonal breeding and growth cycles in the deep sea^{2–5}. Because productivity was not measured simultaneously with our trap collections, and our collections extended over two-month periods, we cannot see what delay there is between the peak in surface productivity and the peak in arrival of particulate matter in deep water. The biweekly measurements of Menzel and Ryther showed peaks in primary production from February to April; our measurements of the particulate flux peak in April/May but the peaks in the deep water may also extend back into March which was not sampled in either year. The data suggest that seasonality in the organic carbon flux is not wiped out at a depth of 3,200 m, indicating rapid settling of the greater part of the organic matter, much of it probably in the form of faecal pellets^{8,12,13}.

The year-to-year variation is of the same order as that observed by Menzel and Ryther⁹, but we may have missed the 1978 peak just before our sampling programme began.

It is possible that the observed fluctuations are due to the trap collecting particles carried in by currents from different directions at different times of the year. If this mechanism operates it does not seem to do so on a very large geographical scale. The material must have come from an area with very similar seasonal pulsation as is shown by the correspondence with the primary production cycle. We have also observed a similar correspondence in the succession of population peaks of planktonic foraminiferal species in the surface water¹⁴ and the arrival of those peaks in the sediment trap. Any lateral influx of particles into the water surrounding the sediment trap does not apparently eliminate the seasonal signal.

The variations in the particle flux shown in Fig. 1 might also have been caused by varying particle trapping efficiency of a uniform flux if there were a seasonally changing current regime at the depth of the trap. Gardner¹⁵ has shown that funnel-shaped traps with baffles at the top, similar to the design used in this study, have an overall trapping efficiency of 70–90% for

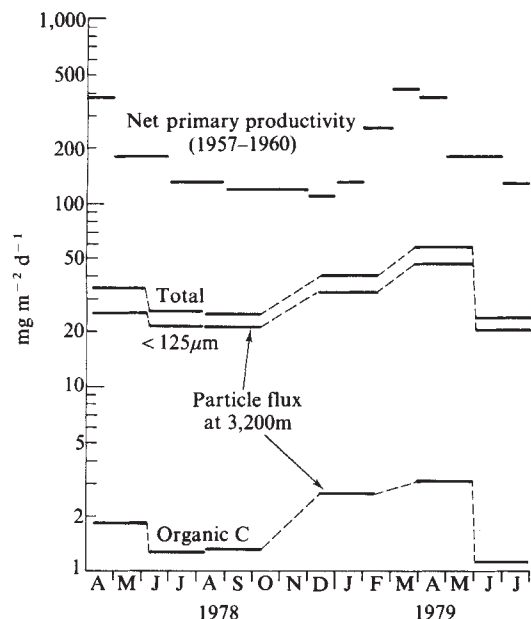


Fig. 1 Comparison of monthly averages of daily net primary productivity (in terms of carbon) for the years 1957–60 (ref. 9) with the average daily yields of total sediment (dry weight) and particulate organic carbon during six recent 2-month sampling periods by a sediment trap. The measurements refer to locations 15 and 25 miles, respectively, south-east of Bermuda. The trap was placed 3,200 m below the surface and 1,000 m above the sea floor.

particles $<25 \mu\text{m}$ in horizontal flows up to 9 cm s^{-1} . For larger particles the efficiency should be greater. Gardner also showed that the efficiency of particle retention in the trap varies with flow speed and angle of attack of the flow. While we have no current data from the mooring site and depth, continuous measurements at a depth of 4,018 m, 27 miles north-northwest of Bermuda (N. Hogg, personal communication) show no indication of a seasonal signal in either speed or direction. The period covered by that measurement series was April 1975 to January 1976. Current speeds were generally $<15 \text{ cm s}^{-1}$ and 70% of the time $<7.5 \text{ cm s}^{-1}$. There is no evidence that the current regime at the trap site should be significantly different. Neither is there any theoretical reason nor experimental evidence for seasonal current changes at that depth anywhere in the North Atlantic. Therefore, we consider it unlikely that the flux variations we have observed are a sampling artefact. We cannot, however, completely dismiss the possibility that the observed fluctuations are random rather than seasonal. Observations over a much longer time span are needed for a proper assessment of that possibility.

It is interesting to compare the range and means of the net primary production⁹ and of the arrival of organic carbon at a depth of 3,200 m at very nearly the same location. Menzel and Ryther measured net primary production in the range 100–800 $\text{mg C m}^{-2} \text{ d}^{-1}$ with a mean around 200 mg . Our measurements at 3,200 m show fluxes ranging from 1 to 3 mg with a mean close to 2 mg . It thus seems that 1% of the net production at the surface penetrates beyond 3,000 m with the small-particle flux, a figure which agrees with many earlier deductions¹⁶ as well as with *in situ* respiration measurements of benthic communities at comparable depths¹⁷.

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Direct measurement of the attenuation of ocean waves by pack ice

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Early experimental work in the Antarctic¹ using a ship-borne wave recorder has shown that pack ice can significantly attenuate incoming ocean waves, particularly those of shorter period. Wadhams^{2,3} has subsequently reported similar wave decay through Arctic ice in data obtained remotely by means of airborne laser profiling and inverted echo sounding from a submarine. The two predominant mechanisms by which the decay can occur—scattering by individual ice floes and energy loss by creep within each flexing floe—have been studied theoretically by Wadhams^{4,5}, Squire^{6,7}, and Squire and Allan⁸. We present here the results of further experiments to measure wave decay which took place in the Bering Sea during spring 1979 from the research ship Surveyor of the National Oceanic and Atmospheric Administration⁹. During the cruise, oceanographic, meteorological and remote sensing data were also collected^{10,11}. The results show the energy decay of waves in pack ice to be exponential with an attenuation coefficient which increases with decreasing wave period.

The attenuation experiment comprised eight floe stations of similar size and thickness, which lay along a line parallel to the direction of the observed principal swell (the attenuation line). They were chosen so that their separation increased away from the ice edge (Fig. 1). The seven floes within the ice pack were visited using the ship's Bell 206 helicopter equipped with a GNS-500A global navigation system which had been pre-programmed from Loran-C on Surveyor. Distances calculated from position fixes should therefore be good to within 0.2 km (Table 1). The first floe, situated on the ice edge itself $\sim 1 \text{ km}$ from Surveyor, was reached using the ship's whaleboat. The heaving motion of each floe was measured using a Schaevitz-EM vertical accelerometer. For logistic reasons we were not able to instrument floes simultaneously so that the complete experiment lasted about eight hours. Ideally such an experiment should take place over a very short time so that waves incident on the ice cover may be assumed to be statistically stationary. To

Table 1 Position of ice floes along attenuation line

Floe station	1	2	3	4	5	6	7	8
Distance from ice edge (km)	0	0.7	2.2	5.3	10.3	18.9	38.6	65.1

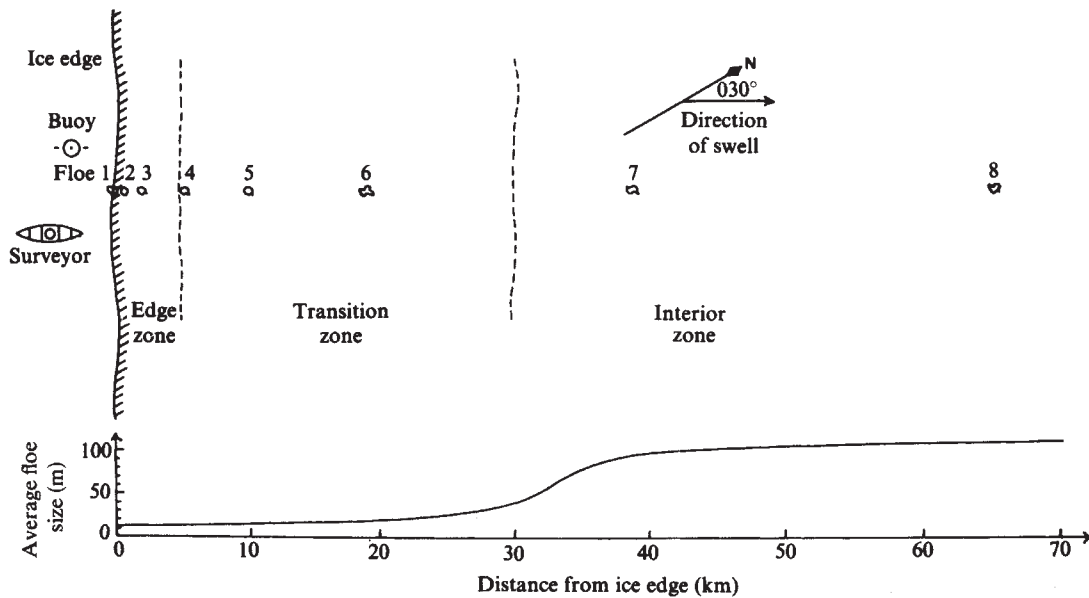


Fig. 1 Schematic diagram of attenuation experiment.

overcome this problem a Waverider buoy was deployed from Surveyor to monitor the open-water wave spectrum.

Routine ice observations were carried out during each helicopter flight. The ice margin was diffuse with an average concentration of four Oktas for tens of kilometres into the ice cover with occasional isolated bands of higher concentration. Floe diameter increased gradually from 10 m at the ice edge to 40 m some 30 km in. At 30 km, the floe size increased abruptly to more than 100 m in diameter, the typical size thereafter. From the air the ice cover could be divided into three distinct zones made up of floes of quite different character: the edge zone, which stretched from the ice edge some 5 km into the pack; the transition zone, which stretched a further 25 km; and the interior zone. This zonal structure had been observed earlier in the field trip and was the reason behind our choice of floe separation. The edge zone was made up of floes which had been buffeted together in waves of sufficient intensity to ridge, raft and fracture the ice. Floes were typically 10 m across, of uneven thickness, with heavily rafted perimeters. Floes within the transition zone were much smoother since the incoming waves had lost much of their energy in the extremely active edge zone. The penetrating waves were still able to fracture the ice, however, and the average floe diameter remained 10 m. Further into the ice cover, the incoming swell had been attenuated to such an extent that little or no fracturing took place and floes in the interior zone were typically smooth and >100 m across.

All the floes visited were between 10 and 20 m across, less than 0.5 m thick and relatively smooth. CTD casts had shown the underlying seawater to be well-mixed and well above the freezing point, and this together with several days of warm weather, had left the ice in a mushy and decaying condition. Twenty minutes of wave data were collected on each of the floe stations and it was noted that there were at least two components

of swell present. There seemed to be a noticeable attenuation between floes but at this stage we could not be sure that the change in amplitude was not due to a decline in the open-water wave forcing. The wave records obtained at each site were digitised and, together with the Waverider buoy data, were calibrated and transferred to IBM digital tape for further analysis. All data processing was carried out on the IBM 370 at Cambridge University using the FFT/graphics package developed by the Sea Ice Group at the Scott Polar Research Institute (SPRI).

The Waverider buoy data are presented in Fig. 2. Each power spectrum represents a 20-min sample of the open-sea wave conditions during the experiment. Records are between 1 and 2 h apart. Within the statistical errors generated by a frequency domain analysis¹², the smoothed power spectra show the wave conditions off the ice edge to be stable throughout the day. This being so, the wave forcing for the attenuation run may be assumed stationary and any comparison between wave data from individual ice floes is valid.

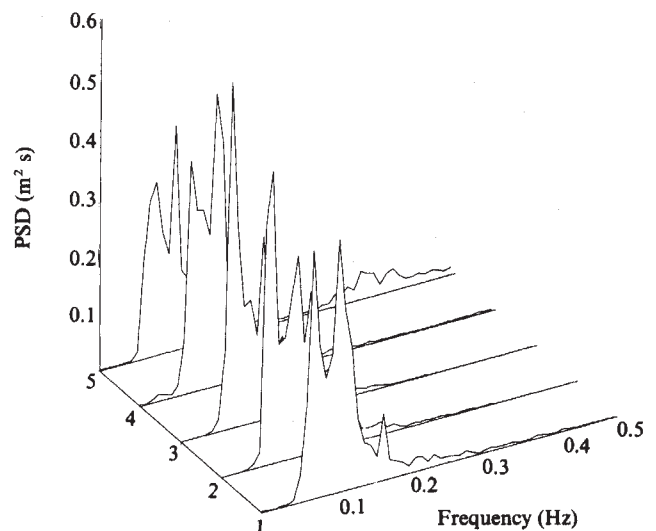


Fig. 2 Three-dimensional graph showing evolution of power spectra from Waverider buoy during the experiment.

Table 2 The attenuation coefficients for energy decay at various central wave periods

Central period (s)	Attenuation coefficient ($\text{m}^{-1} \times 10^{-4}$)
12.2	0.272 ± 0.054
9.4	0.438 ± 0.036
7.6	0.855 ± 0.049
6.4	1.087 ± 0.037
5.5	1.214 ± 0.192

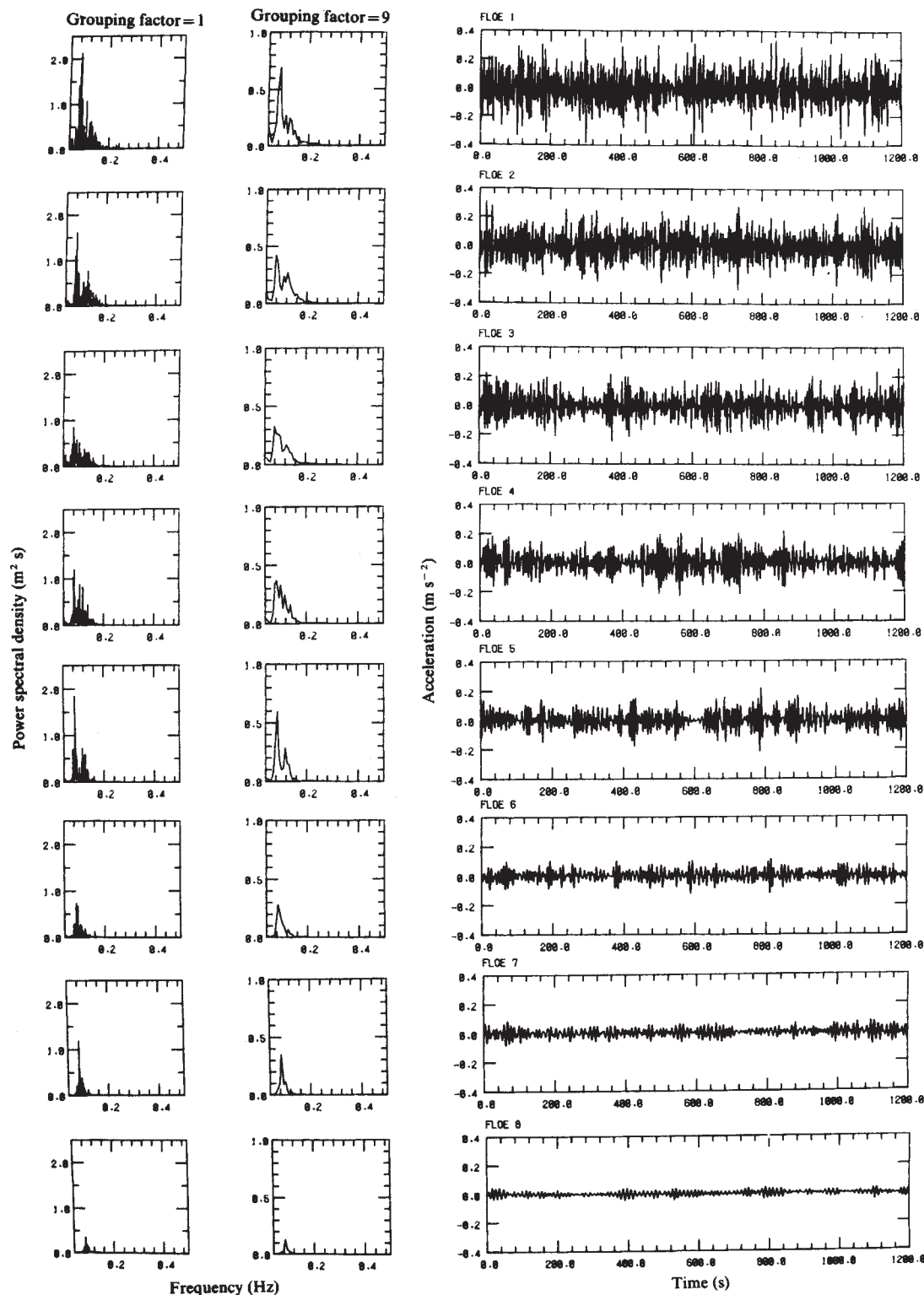


Fig. 3 The attenuation run showing time series at each floe and corresponding power spectra.

Figure 3 shows the time series and the unsmoothed and smoothed power spectra for the wave data recorded at each floe station. All records are 20 min (1,200 s) long and are plotted to the same scale so that a direct comparison may be made. Each time series represents the vertical acceleration at a point along the attenuation line. There is a clear attenuation with distance from the ice edge together with a noticeable filtering of the incoming waves in favour of longer wavelengths. The power

spectra confirm these observations. Each pair of spectra, derived from the corresponding time series and corrected for acceleration, shows the power spectral density (PSD) as a function of frequency. The first spectrum of the pair is unsmoothed and is presented for completeness. The second has been frequency smoothed with a grouping factor of nine. There is a striking decay in the available wave energy as the reader proceeds from floe to floe. Furthermore,

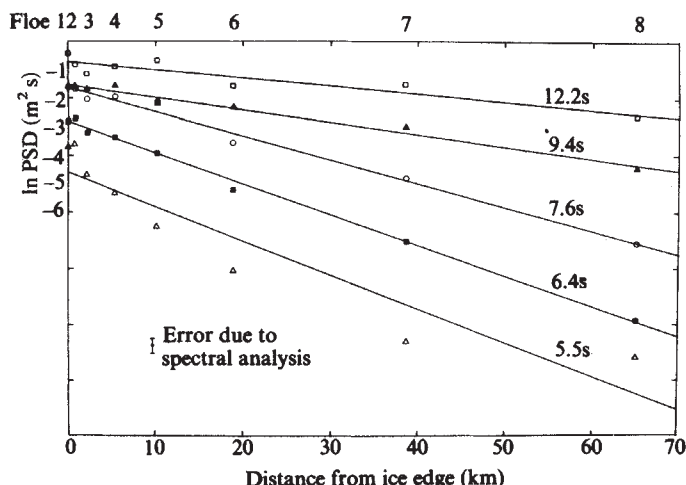


Fig. 4 Energy decay as a function of wave period.

waves of shorter period are attenuated more rapidly than long waves.

From the smoothed spectra, five central wave periods were selected at which significant wave energy was present. Figure 4 shows these wave energies plotted against distance from the ice edge. The energy decay is seen to be approximately exponential with the attenuation coefficient a function of wave period (Table 2). The line corresponding to a period of 5.5 s shows the most scatter. This is believed to be due to an increase in energy at shorter periods occurring late in the day (see Fig. 2). If the 5.5 s point for floe 8 is assumed to be an overestimate and is neglected, the best straight line between the remaining seven stations gives an attenuation coefficient of $(1.824 \pm 0.183) \times 10^{-4} \text{ m}^{-1}$. With this revised value for the energy decay at 5.5 s, the data fit the empirical inverse square relationship between attenuation coefficient and wave period found by Wadhams². Similar studies carried out off the coast of East Greenland¹³ by SPRI, the Cavendish Laboratory at the University of Cambridge, and the Technical University of Denmark promise attenuation runs for thicker floes and longer wave periods.

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KBS Tuff dating and geochronology of tuffaceous sediments in the Koobi Fora and Shungura Formations, East Africa

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Plio-Pleistocene lacustrine and fluvial sediments of the Koobi Fora Formation along the east shore of Lake Turkana in northern Kenya (see Fig. 1) and thicker fluvial sediments of the Shungura Formation in the Lower Omo River Basin 100 km to the north have become widely recognised because of their rich hominid and artefact assemblages, together with associated vertebrate fauna^{1–3}. Over the past decade, tuffaceous horizons within these two sedimentary formations have been mapped^{4,5} and dated by K–Ar^{6–11} and fission-track techniques¹² to provide chronologic control supporting studies of hominid and faunal evolution and archaeology. We report here new ⁴⁰K–⁴⁰Ar dates and revised values for previously published dates which give a mean age of 1.8 ± 0.1 Myr for the KBS Tuff. This estimate suggests contemporaneity between the KBS Tuff and Tuff Units H2 and H4 in the Shungura Formation, lower Omo River Basin, Ethiopia, and with Bed I at Olduvai Gorge.

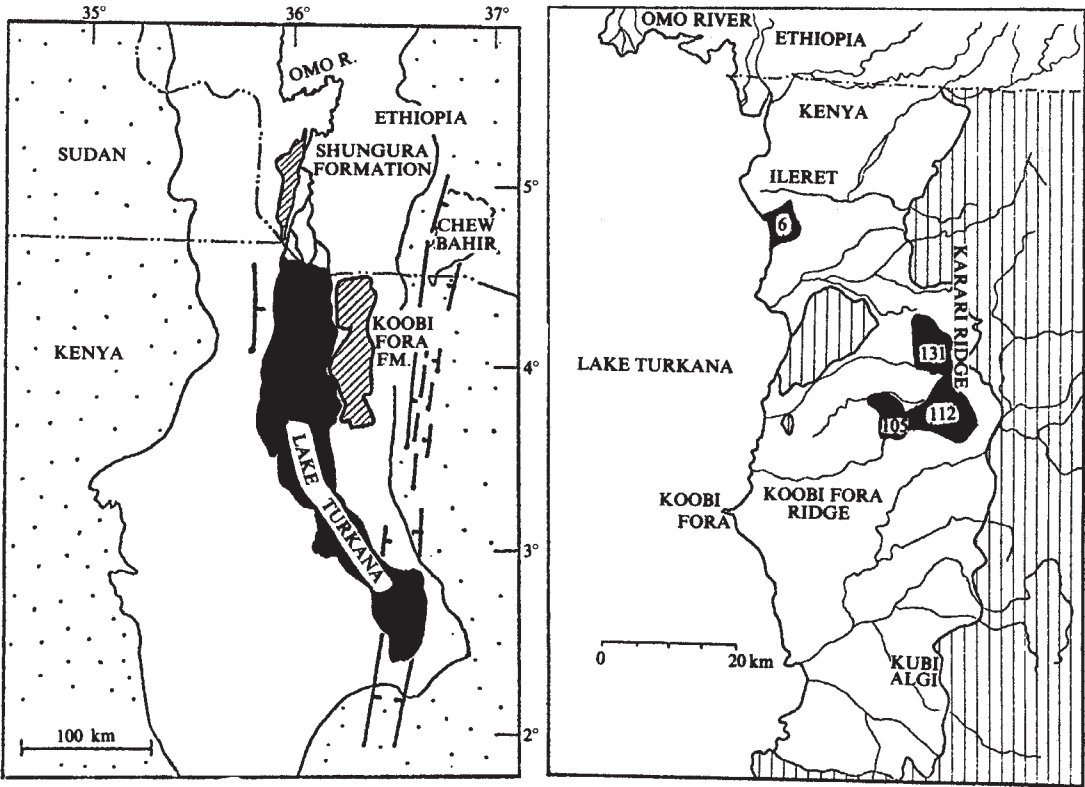
Although isotope dates for most of these tuffaceous horizons have been stratigraphically and palaeontologically consistent, some have remained uncertain. Of these, the KBS Tuff of the Koobi Fora Formation has been the most controversial, particularly because of its importance in placing an upper age limit on the important hominid skull KNM-ER-1470 with its associated vertebrate fauna, and on artefacts embedded in the tuff^{13,14}.

In 1975, we reported ⁴⁰K–⁴⁰Ar dates indicating the KBS Tuff and its correlative horizons contained at least three pumice populations with two different ages⁷. All dates from Area 105 (see Fig. 1) averaged 1.6 Myr, while those from Area 131 averaged 1.8 Myr. Both dates were significantly younger than the previously assigned age of 2.61 Myr based on a ⁴⁰Ar–³⁹Ar incremental heating date⁶. Even though the 2.61 Myr date was subsequently revised to 2.42 Myr (ref. 10), in apparent agreement with newly obtained fission-track dates on zircons separated from KBS pumices¹², a substantial age discrepancy still remained between our conventional ⁴⁰K–⁴⁰Ar results and those obtained by ⁴⁰Ar–³⁹Ar and fission-track dating techniques.

To resolve this problem, we dated a new group of KBS pumice samples that were collected in 1977. All of these new dates fell in the 1.8 Myr range, and none reproduced our previous 1.6 Myr dates. This result led to the discovery of a systematic error in potassium analyses for all KBS samples which had a given 1.6 Myr date. This error was traced to a defective balance. Recalculation using revised potassium contents obtained by re-analysis of the originally dated material increased all of the 1.6 Myr dates to 1.8 Myr, bringing them into agreement with the new dates, and completely eliminating the evidence of any KBS pumice 1.6 Myr old (see Fig. 2.)

With this revision, we now have 22 KBS dates; 16 on feldspars and 6 on glasses that were separated from a total of 13 different pumice samples collected within the KBS Tuff in Areas 131, 105 and 112. Fig. 2 shows that statistically, these dates give a mean of 1.8 ± 0.1 Myr at the 95% confidence level. For correlation and interpretation, the dates of all of the pumices so far sampled must be treated as being indistinguishable in age, even though several geochemically distinct pumice types representing separate eruptive events occur together in the KBS Tuff horizon. As we can distinguish dates that are >100,000 yr apart,

Fig. 1 Maps showing sample localities in the Koobi Fora Formation east of Lake Turkana and their geographic relation to the Shungura Formation in the lower Omo River Basin.



it seems reasonable to assume that several eruptive events which led to the accumulation of the various pumice fragments in the KBS Tuff took place within this time interval, and may contribute to the scatter of dates about the mean.

In 1970, the first K-Ar dates from the KBS Tuff were obtained on feldspars by Fitch and Miller using ^{40}Ar - ^{39}Ar incremental heating and total degassing procedures⁶. Further work produced dates ranging from 0.52 to >200 Myr (ref. 8). As

Fitch and Miller recognised, the extremely old dates resulted from detrital contamination with ancient crystalline basement rocks. Disregarding those old dates, one is still faced with a broad range of possible dates from which 2.61 ± 0.26 Myr was originally selected as the 'best apparent age' of the KBS Tuff. The subsequent revision to 2.42 ± 0.01 Myr using a different J value¹⁰ did not reduce this scatter. We feel that the apparent discrepancy between our ^{40}K - ^{40}Ar results and the date favoured

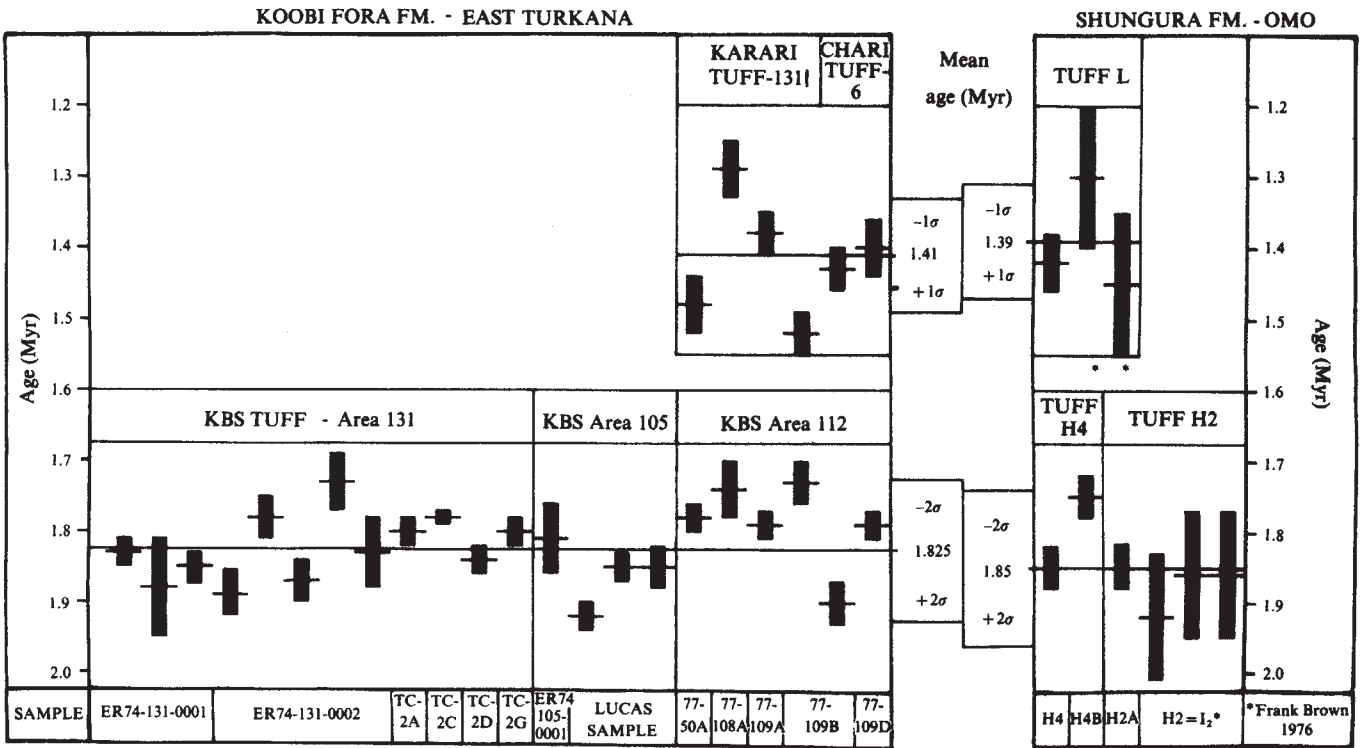


Fig. 2 Age correlations of KBS Tuff with Tuff Units H₂ and H₄ and the Karari-Chari Tuffs with tuff L.

Table 1 K-Ar analytical data

KA No.	Material	Sample wt (g)	% K	^{40}Ar radio- genic ($\times 10^{-11}$ mol g^{-1})	% ^{40}Ar atmospheric	Age (Myr)	Sample	Location
2836	sanidine	4.877	5.10*	1.62	36	1.83 ± 0.02	KBS ER74-131-0001	Area 131
2837	glass	3.477	3.92	1.28	93	1.88 ± 0.07		
2869	sanidine	2.156	4.60	1.47	53	1.85 ± 0.02		
2841	sanidine	1.945	4.90*	1.61	52	1.89 ± 0.03	KBS ER74-131-0002	
2842	sanidine	3.002	5.11	1.58	41	1.78 ± 0.03		
2843	glass	3.514	3.48	1.13	83	1.87 ± 0.03		
2858	glass	2.870	3.69*	1.11	81	1.73 ± 0.04		
2862	glass	2.778	3.73*	1.18	83	1.83 ± 0.02		
3160	sanidine	6.266	5.09	1.59	30	1.80 ± 0.02	KBS TC-2A	
3166	sanidine	3.321	5.04	1.56	26	1.78 ± 0.01	KBS TC-2C	
3161	sanidine	3.493	5.12	1.64	37	1.84 ± 0.02	KBS TC-2D	
3167	sanidine	3.019	4.87	1.61	36	1.90 ± 0.02	KBS TC-2G	
2851R	sanidine	1.940	5.55*	1.74	39	1.81 ± 0.03	KBS ER74-105-0001	Area 105
2856	sanidine	1.736	4.70*	1.57	37	1.92 ± 0.02	KBS Fred Lucas sample	Area 105E
2854	glass	3.558	3.89*	1.25	53	1.85 ± 0.02		
3496	sanidine	2.253	5.11	1.64	73	1.90 ± 0.03		
3182	sanidine	2.422	5.19	1.61	42	1.78 ± 0.02	KBS 77-50A	Area 112
3189	sanidine	3.504	5.08	1.54	74	1.74 ± 0.04	KBS 77-108A	'Hippo site'
3188	sanidine	2.699	4.96	1.54	33	1.79 ± 0.02	KBS 77-109A	Area 112
3186	sanidine	4.438	4.87	1.46	59	1.73 ± 0.03	KBS 77-109B	
3380	glass	3.010	4.04	1.33	51	1.90 ± 0.03	KBS 77-109B	
3187	sanidine	6.142	5.12	1.59	30	1.79 ± 0.02	KBS 77-109D	
2970	sanidine	2.339	5.06	1.62	45	1.85 ± 0.03	H ₄	Omo
3183	sanidine	1.143	5.09	1.54	60	1.75 ± 0.03	H _{4B}	
3256	sanidine	4.291	4.93	1.59	48	1.85 ± 0.03	H _{2A}	
3254	sanidine	2.322	3.94	0.96	71	1.42 ± 0.04	L	
2815	sanidine	4.264	4.05	1.04	77	1.48 ± 0.04	Karari 108-000	Area 131
2863	sanidine	1.676	4.15	0.93	82	1.29 ± 0.04	Karari 108-0003	
2882	sanidine	2.748	4.24	1.12	36	1.52 ± 0.03	Karari 108-0002	
2880	sanidine	3.161	4.08	0.97	41	1.37 ± 0.03	Karari 108-0004	
2865	sanidine	1.718	3.92	0.94	59	1.38 ± 0.03	Chari 006-0001	Area 6
2883	sanidine	1.010	3.89	0.94	75	1.40 ± 0.04	Chari 006-0120A	

Decay constants: $\lambda_{40\text{K}\beta} = 4.962 \times 10^{-10}$ yr; $\lambda_{40\text{K}\alpha} + \lambda'_{40\text{K}\alpha} = 0.581 \times 10^{-10}$ yr

Isotopic abundance: $^{40}\text{K} = 0.01167\%$ K_{total}

* K content reanalysed and date recomputed from 1975 published results. All 1975 dates have been recalculated using the above constants.

by Fitch and Miller arises through their selection of a particular value from a scatter. Their criteria used for selecting this date are debatable. Based on their experience of interpreting ^{40}Ar - ^{39}Ar release patterns of plutonic minerals known to have been thermally overprinted, they believed they could not only see effects of similar overprinting of feldspar from KBS pumices, but could also infer a geological age for these events. Such an interpretation for KBS pumice dates is not supported, however, either by our data or by theirs. Furthermore, many of those with ^{40}Ar - ^{39}Ar dating experience support the conclusions of Lanphere and Dalrymple¹⁵ that "it may be difficult or impossible to extract geologically meaningful information from the age spectrum of a disturbed sample."

We have demonstrated the repeated concordancy of dates on glass and feldspar taken from the same pumice clasts (Table 1). As glass devitrifies and loses argon much more readily than sanidine or anorthoclase feldspar, the concordancy of dates argues strongly against thermal overprinting. Such overprinting would manifest in significantly younger dates for glass with respect to coexisting feldspar.

To interpret Fitch and Miller's ^{40}Ar - ^{39}Ar incremental heating data following a thermal overprinting model requires a different history of thermal events for almost every sample dated. Only in this way can many otherwise reasonable KBS dates be disregarded. (In fact, several of Fitch and Miller's better total degassing dates and at least one ^{40}Ar - ^{39}Ar age spectrum support a 1.8 Myr age.) The inability to obtain reproducible results apparently led to special interpretations which are not satis-

factorily supported by the data presented. We feel, therefore, that the 1.8 Myr mean of our tightly clustered ^{40}Ar - ^{40}K dates provides a better estimate of the age of the KBS Tuff than the ^{40}Ar - ^{39}Ar dates selected by Fitch and Miller.

Another aspect of the dating problem was brought to light by fission-track dates on zircons¹² from two KBS pumices which gave a mean of 2.44 ± 0.08 Myr, apparently supporting the ^{40}Ar - ^{39}Ar age advocated by Fitch and Miller¹⁰, but differing from our 1.8 Myr ^{40}K - ^{40}Ar mean of 1.8 Myr.

Although the mean of five dates yielded this statistically precise value, the individual dates have much larger error estimates because fission-track counting was done only on a few zircons which have very low track densities. Hence, any minor systematic error in technique could greatly bias the age, and a statistically derived estimate of precision may not be relevant to the accuracy of the mean of these dates. If this is so, the apparent discrepancy between our ^{40}K - ^{40}Ar mean date of 1.8 Myr and the fission-track mean may be resolved by increasing the fission-track confidence interval to express the possibility that systematic errors may have yielded an excessive age. This apparent discrepancy between the fission-track age estimates of Hurford, Gleadow and Naeser¹² and the mean of our 22 tightly clustered ^{40}K - ^{40}Ar dates deserves investigation, but for the present we contend that our mean date is an age estimate with much more clearly defined confidence limits than the fission-track age.

The mean value of 1.8 ± 0.1 Myr for the KBS Tuff and a mean value of 1.4 ± 0.1 Myr for the Chari-Karari Tuffs of the Koobi

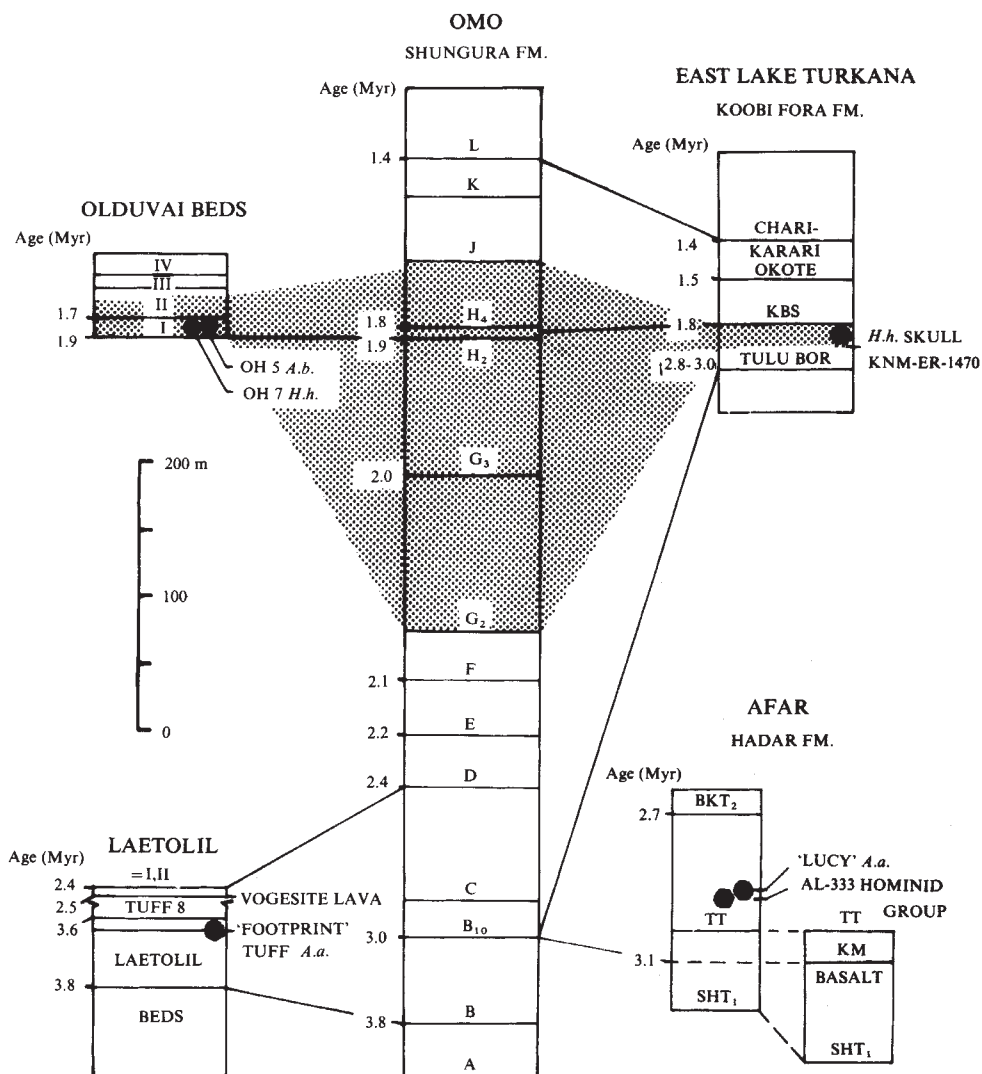


Fig. 3 Comparison of dated stratigraphic sections of the Koobi Fora Formation, Shungura Formation, Laetolil Beds, and the Hadar Formation in East Africa. The stippled area represents biostratigraphically contemporaneous units.

Fora Formation are indistinguishable from K-Ar dates on Tuff Units H₂ and H₄ and Tuff Unit L¹¹ respectively in the Shungura Formation (Fig. 2).

The results of extensive geochemical and geophysical analyses of pumices and tuffaceous matrix from these tuffs¹⁹ have enabled the identification of unique pumice types in the KBS-H₂, H₄ horizons that are probably derived from the same eruptive event though they occur in sedimentary sections 100 km apart. As the Shungura Formation has yielded a long and consistent sequence of ⁴⁰K-⁴⁰Ar dates, this independent geochemical correlation of the KBS Tuff and Tuff Units H₂ and H₄ strengthens the case for a 1.8 Myr age for the KBS Tuff.

Figure 3 compares the generalised stratigraphic sections and dated horizons within the Olduvai Beds, the Shungura Formation, the Koobi Fora Formation, the Laetolil Beds, and the Hadar Formation to show the relationship of the ⁴⁰K-⁴⁰Ar time scales that have been established for each stratigraphic section^{11,16-18}.

Cooperative studies of the taxonomic composition of fossil vertebrate assemblages in the beds immediately below the KBS Tuff, between Tuffs G₁ and J in the Shungura Formation and in Bed I at Olduvai Gorge suggest that all three are biostratigraphically indistinguishable units^{1,2} as shown by the stippled area of Fig. 3. Further detailed studies of the stage of evolution of certain rapidly changing lineages, especially among the Suidae also suggest contemporaneity (see refs 20, 21).

Bed I at Olduvai Gorge has been dated by numerous ⁴⁰K-⁴⁰Ar dates as between 1.7 and 1.9 Myr (ref. 17). Member G between Tuff Units G and H in the Shungura Formation is dated by

⁴⁰K-⁴⁰Ar as between 1.8 and 2.0 Myr (ref. 11). These beds and those below the KBS Tuff exhibit a palaeomagnetic stratigraphy consistent with an Olduvai event age between 1.76 and 1.91 Myr (refs 22, 23). We consider it unlikely, therefore, that the KBS samples which also yield the same ⁴⁰K-⁴⁰Ar dates as the Olduvai and Omo localities could have been subjected to overprinting or other disturbances as are postulated by Fitch and Miller.

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Wind velocities determined from the surface textures of sand grains

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Krinsley and Smalley¹ suggested that 'upturned plates', a surface texture that is represented on quartz sand surfaces by a series of thin parallel plates, either continuous or discontinuous parallel to $r(10\bar{1}1)$ and $z(01\bar{1}1)$, cause the frosting observed on desert sand grains. As these plates depend on the extent of physical abrasion², the spacing between individual plates might be related to the velocity of the wind which last transported the grains. The technique described here attempts to relate experimental and modern aeolian sand grain surface textures to wind velocity via scanning electron microscopy (SEM)³.

To test this hypothesis, we examined crushed Brazilian quartz abraded in a paddle wheel device at four effective wind velocities: 8, 24, 32 and 40 m s⁻¹ (ref. 4). Two modern Saharan desert sands where the average wind velocity was known from meteorological data, and a Permian eolian sand were also studied. The paddle wheel apparatus was driven by a variable speed motor that moves particles at a given rate and causes particle self abrasion; the apparatus was constructed to simulate aeolian action on Mars, as previous devices could not operate at the low pressures and high velocities presumably present on Mars⁴. At normal pressures and velocities, surface textures on experimental sand grains were identical to those produced by other devices used to simulate natural aeolian processes⁵. Upturned plates on grains were photographed using SEM; stereo photogrammetry was then used to measure separation between the plates.

Plate spacing measurements on the experimental samples (175–355 µm diameter) exhibit three significant characteristics: (1) spacing is constant across individual grain surfaces; (2) spacing on grains abraded at similar velocities is reasonably constant; and (3) grain size has little effect on spacing (two grain sizes averaging 200 and 300 µm diameter were used).

Plate spacing on the 20 experimentally produced aeolian grains was measured using stereo microscopy (Table 1). The results of a regression analysis of the plate spacing (y) plotted against the wind velocity (x) for this experimental material (Fig. 1) show that the best fit ($r = 0.94$) line for these data is described by the polynomial expression:

$$y = 0.000032x^3 - 0.0017x^2 + 0.0364x + 0.0016 \quad (1)$$

The curve representing this equation is characterised by three zones (delineated by two slope changes) which were observed in the Sahara desert^{6–8}.

The first zone (from 0 to 7 m s⁻¹) is the velocity range in which grains have lost all of their sharp edges and have begun to round. The presence of frosting on edges indicates that these areas were the most susceptible to abrasion. Sand grains at this range of wind velocities have been observed to be mobile for only short distances in deserts.

The second zone extends from 7 to 27 m s⁻¹; the flattening of the curve here suggests that physical changes due to abrasion are linearly related to wind velocity. The calculated average daily

wind velocity is from 5 to 8 m s⁻¹ in the Sahara⁶ and it has been observed that sand clouds form and sand actively migrates across the desert at velocities of 10–13 m s⁻¹; sandstorms begin to form at velocities from 13.7 to 15.7 m s⁻¹ (refs 7, 8). Note that the maximum continuous wind velocities observed in the Sahara are represented in this zone.

The third zone (no measurements have been continuously recorded above 27 m s⁻¹ shown on the curve ranges from 27 to 40 m s⁻¹. Here the slope increases very rapidly, suggesting rapid physical changes on sand grain surfaces; these have been observed with SEM on individual sand grains abraded at the two highest velocities (32 and 40 m s⁻¹). In this range, grain size seems to decrease rapidly; the grains become more spherical, and plastic deformation and/or partial melting may occur on surfaces⁴.

Micron-sized quartz particles, after abrasion at 27–40 m s⁻¹, are structurally deformed and SEM observations indicate that rounding and smoothing occur on quartz sand grains at these velocities⁴. Calculations also show that the collision between two quartz grains at 15 m s⁻¹ would raise the temperature of a microscopic feature (~5 µm diameter) on their surfaces to above the melting temperature of quartz⁴.

Extrapolation of the curve past 40 m s⁻¹ suggests that it becomes asymptotic at some higher velocity. This indicates that there may be a limit or terminal velocity where either grains achieve total sphericity or break down into silt and clay-size fragments. The large population of fine silt-sized debris in the 40 m s⁻¹ sample suggests that this limit probably results in grain destruction ('kamikaze grains' as in ref. 9), and not perfect sphericity. Note that when the fine silt particles from the 40 m s⁻¹ sample are studied at high magnification, some of them exhibit a finely abraded and rounded surface while other surfaces display conchoidal fracture. Thus, these particles may represent portions of rounded and frosted grains which subsequently have been destroyed during the experiment.

Photomicrographs of 10 modern Saharan dune sand grains were studied (Table 2) first to determine if plate spacing could be measured on naturally occurring sand grains, and second to obtain a reasonable approximation of the maximum wind velocity which last moved them.

The Libyan grains fall into the lowest velocity zone; they were collected from an inactive interdune area. Solution and/or precipitation and small bits of debris sticking to grain surfaces suggest that the grains were not being actively abraded at the time of collection. The wind conditions of this sub-environment (5–8 m s⁻¹, (ref. 8)) are comparable to those calculated directly from aeolian plate spacings (6.33 ± 0.04 m s⁻¹, Table 2).

The Algerian sand was collected from an active dune. Here, solution and/or precipitation features can be recognised only in depressions on the grains with clean surfaces indicating that they have undergone recent active abrasion. The calculated wind velocity for this sample (13.53 ± 0.05 m s⁻¹) lies well within the values observed by Bagnold for actively saltating grains (10–15.7 m s⁻¹).

Plate spacing on Permian aeolian sands was then examined (Table 2). The Permian sandstone deposits of County Durham, UK have been interpreted as a hot, dry, Saharan type of aeolian deposit¹⁰. Minor quartz overgrowths and precipitation features are present, but do not obscure grain surfaces. Some of these features may even aid plate measurement; the aeolian plates seem to act as a growth site for secondary quartz. Measurements

Table 1 Measured average aeolian plate spacings from experimentally abraded Brazilian quartz grains

Velocity (m s ⁻¹)	No. of grains	No. of measurements	Grain size range (µm)	Average spacing (µm)
8	5	98	175–355	0.207 ± 0.041
24	5	78	175–355	0.338 ± 0.048
32	5	88	175–355	0.490 ± 0.073
40	5	71	175–355	0.777 ± 0.130

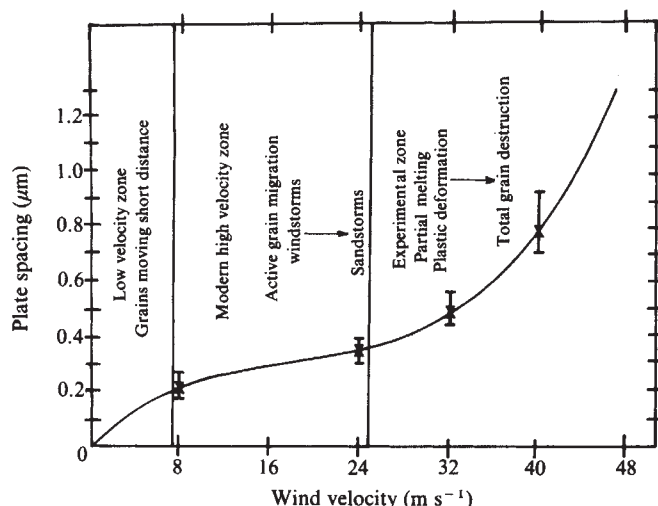


Fig. 1 Comparison between changes in slope of experimental data and wind velocity for various types of sand grain movements observed in deserts.

of plate spacing were substituted in the regression equation; the calculated wind velocity is 29 m s^{-1} , a value slightly higher than the strongest continuous wind velocities observed in the modern Sahara (22 m s^{-1} (ref. 6)). If the technique is valid, these sands could have been transported and deposited in a hot arid Saharan type desert by winds of higher velocities than those in the modern Sahara.

If the time required to remove a thickness equal to the relief of the plates is short enough, the spacing measured will represent the final abrasion episode. If the time is measured in days, weeks, or months, then the spacing must represent some average value related to the velocity of a number of storms.

Crushed Brazilian quartz averaging $\sim 500 \mu\text{m}$ in diameter was abraded at a velocity of 8 m s^{-1} for three hours in the paddle wheel device. The grains were then removed and studied with the scanning electron microscope; $>80\%$ of all surfaces were covered with small abrasion markings. Upturned plates constituted a large portion of the abraded surfaces. Three hours is probably the maximum abrasion time necessary to cover almost completely the surface with abrasion markings; the only remaining non-abraded areas were those that were at least $5 \mu\text{m}$ below the general surface and thus were protected from immediate abrasion. Although the original starting material was fairly large and quite angular, the experiment suggests that times of the order of minutes or hours in a sandstorm would resurface natural sand grains.

Additionally, 500 g of natural quartz sand (sieved to between 125 and $180 \mu\text{m}$) was fed into an aeolian whirling arm device¹¹ and used to abrade, in an aeolian mode, six quartz flats at 20 m s^{-1} . The sand stream struck the targets perpendicular to their surfaces for 23 min with 16 g of sand actually striking each target. Note that each grain of sand struck each flat only once. Finally the targets were weighed to an accuracy of five places before and after bombardment.

The time needed to remove completely a layer of quartz the height of an average plate ($0.5 \mu\text{m}$) was calculated and was 29 min . This is probably the maximum time needed to remove a significant fraction of the old plates and produce new ones; thus in a very short time a high velocity wind could reset the spacing over a large portion of grain surfaces. The experiment, of course, was conducted with a fixed target; natural aeolian grains are round and rotate, which would tend to create an isotropically eroded surface similar to the experimental targets. The two experiments give order of magnitude results, but even if the final answer were three times as large, plate spacing on aeolian grains probably represents the final windstorm velocity to which the grains were subjected.

Table 2 Sample locations, plate spacing and calculated wind velocities for Saharan and Permian sands

Sample location	No. of grains	No. of measurements	Average spacing (μm)	Calculated wind velocities (using equation (1))
Sehba Sand Sea, Libya (Sahara desert)	5	92	0.172 ± 0.045	$6.33 \pm 0.04 \text{ m s}^{-1}$
Great Western Erg, Algeria (Sahara desert)	5	102	0.262 ± 0.039	$13.53 \pm 0.05 \text{ m s}^{-1}$
Permian sandstone County Durham, UK	5	100	0.354 ± 0.047	$29.34 \pm 0.04 \text{ m s}^{-1}$

We stress that plate spacing results from the last abrasion event that occurred on a given sand grain; a change in velocity is assumed to eliminate previous plate spacing imprints and to superimpose the most recent velocity spacing on surfaces. The ability to determine ancient wind velocities could aid further understanding of the paleogeography and paleoclimatology of the vast continental aeolian sand deposits preserved in the geologic record. This research was supported by NASA consortium agreement 2-OR035-801 and 2-OR035-901, Office of Planetary Geology. We thank Paul Spudis and Alan Peterfreund for reviewing this manuscript, also Michael Malin and Steve Williams for technical assistance.

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Why do Bedouins wear black robes in hot deserts?

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Survival in hot deserts has always posed a problem for man; Moses had to solve it in order to lead the children of Israel through the wilderness of the Sinai—a formidable hot desert. It seems likely that the present inhabitants of the Sinai, the Bedouins, would have optimised their solutions for desert survival during their long tenure in this desert. Yet, one may have doubts on first encountering Bedouins wearing black robes and herding black goats. We have therefore investigated whether black robes help the Bedouins to minimise solar heat loads in a hot desert. This seemed possible because experiments have shown that white hair on cattle^{1,2} and white feathers on pigeons³ permit greater penetration of short-wave radiation to the skin than black. In fact, more heat flowed inward through white pigeon plumage than through black when both were exposed to simulated solar radiation at wind speeds greater than 3 m s^{-1} (ref. 3). We report here that the amount of heat gained by a Bedouin exposed to the hot desert is the same whether he wears a black or a white robe. The additional heat absorbed by the black robe was lost before it reached the skin.

Table 1 Radiation exchange and convective heat loss

	Radiation gain	Radiation loss	Net radiation gain	Convective loss	Net radiation gain-convective loss
Black robe	838 ± 10	543 ± 2	295 ± 8	57 ± 7	238 ± 12
White robe	649 ± 3	534 ± 3	115 ± 2	31 ± 5	84 ± 6
Tan army uniform	644 ± 3	452 ± 2	192 ± 2	45 ± 3	147 ± 4
Shorts (semi-nude)	624 ± 10	442 ± 6	182 ± 5	-2 ± 4	184 ± 7

The rate of net heat gain by radiation was more than 2.5 times as great in the black robe as in the white. The greater heat loss by convection from the black robe due to its higher surface temperature was not great enough to compensate for its higher net radiation gain. This leads us to postulate that additional heat was lost by convection through the robes through a bellows effect or a chimney effect. Values in W m^{-2} are the means of nine experiments ± s.e.

To quantify the effects of the colour of a robe on the heat load imposed by a hot desert, we measured and/or calculated the net heat gain by radiation, heat loss by convection, heat loss by evaporation, heat storage and metabolic heat production of a man standing facing the Sun in the desert at mid-day while he wore: (1) a black Bedouin robe; (2) a similar robe that was white; (3) a tan army uniform; and (4) shorts (that is, he was semi-nude). We used one individual as a calibrated heat exchanger, because we were interested in the effects of the coverings rather than in physiological differences among individuals. Robes were purchased from Bedouins and we used two layers of robes in the same manner as they did. Only the outer robe was changed when comparing black and white robes. We selected experimental conditions where both solar radiation and air temperature were high. The measurements were made during the summer at mid-day (1030–1430 h) at the Hatzeva Field School (in the

Rates of metabolic heat production ($\dot{H}_{\text{metab}}$), evaporative heat loss ($\dot{H}_{\text{evap}}$), and changes of heat content of the body ($\dot{H}_{\text{stor}}$) were measured, then net heat gain by non-evaporative means ($\dot{H}_{\text{gain}}$) was calculated by applying the first law of thermodynamics where:

$$\dot{H}_{\text{gain}} = \dot{H}_{\text{evap}} + \dot{H}_{\text{stor}} - \dot{H}_{\text{metab}} \quad (1)$$

All measurements were made during 30-min intervals. The techniques and their accuracies were the same as we have described in detail for similar measurements on the Bedouin's black goats⁵.

The absorptivity of the black robes for radiation in the visible part of the spectrum (0.89) was more than 2.5 times as great as that measured for the white robe (0.35) and about 1.5 times as great as for the army uniform (0.72) or the semi-nude subject (0.66). The greater rate of absorption of visible radiation by the

Table 2 Avenues of heat gain and loss of a subject facing the Sun in a hot desert at mid-day and in a temperature-controlled room at 48 °C

	$\dot{H}_{\text{gain}}$	=	$\dot{H}_{\text{evap}}$	+	$\dot{H}_{\text{stor}}$	-	$\dot{H}_{\text{metab}}$	T_{amb}
Desert								
Black robe	142 ± 4	=	199 ± 4	+	10 ± 1	-	67 ± 2	59.5
White robe	134 ± 11	=	191 ± 8	+	8 ± 1	-	65 ± 2	58.6
Tan army uniform	161 ± 7	=	217 ± 7	+	10 ± 1	-	66 ± 2	-
Shorts (semi-nude)	208 ± 7	=	264 ± 8	+	8 ± 1	-	64 ± 2	62.2
Temperature-controlled room at 48 °C								
Black robe	66 ± 7	=	126 ± 6	+	7 ± 2	-	67 ± 2	
White robe	65 ± 9	=	123 ± 9	+	6 ± 1	-	64 ± 3	
Shorts (semi-nude)	86 ± 9	=	144 ± 9	+	9 ± 2	-	67 ± 2	

Rates of net heat gain ($\dot{H}_{\text{gain}}$) from the environment, evaporative heat loss ($\dot{H}_{\text{evap}}$), heat storage ($\dot{H}_{\text{stor}}$) and metabolic heat production ($\dot{H}_{\text{metab}}$) are given in W m^{-2} and are the means of nine experiments ± s.e. To determine the temperature in our environmental room (T_{amb}) that would be necessary to simulate conditions of our hot desert at mid-day, $\dot{H}_{\text{gain}}$ was quantified when the room was set approximately 10 °C above average skin temperature. It was then assumed that $\dot{H}_{\text{gain}}$ would be directly proportional to the temperature gradient between the skin and room in order to calculate the room temperature required to obtain the same $\dot{H}_{\text{gain}}$ as we obtained in the hot desert.

Negev Desert at the bottom of the rift valley between the Dead Sea and the Gulf of Elat). During the measurements air temperatures ranged between 35 °C and 46 °C (average 38 °C) and wind speed between 0 and 4.1 m s^{-1} (average 1.3 m s^{-1}).

Radiation exchange and heat loss by convection were calculated according to the procedures outlined for cattle⁴ and goats⁵ with the following minor modifications. (1) Swinbank's empirical formula⁶ was used for calculating long-wave radiation from the sky; (2) surface area was calculated from Dubois' formula⁷ when the subject wore the army uniform or was semi-nude (this area was multiplied by 0.85 when calculating diffuse radiation exchanges to correct for exchanges between the legs, arms and body); and (3) surface area for the subject wearing the robes was calculated by assuming he approximated a cylinder whose circumference was measured around the chest.

black robes was not accompanied by a greater rate of radiation loss (Table 1). Although convective heat loss from the black robe was greater than that from the white because of its higher surface temperature, the net inward flow of heat through the black robe (that is, net radiation gain-convective loss) was calculated to be almost three times as great as through the white (Table 1).

In spite of the greater net heat gain by the black robe, there was essentially no difference in rates of net heat gain ($\dot{H}_{\text{gain}}$) by the subject wearing black or white robes (Table 2). Rates of evaporative heat loss, heat storage, and metabolic heat production were essentially the same in the black and white robes.

How does one reconcile these seemingly conflicting results? The explanation must be greater convective transfer of heat through the air spaces beneath the black robe, either by a

bellows action as the robes flow in the wind or in a manner analogous to the functioning of a chimney where the chimney in this case would be the air space between the robes and the skin. The air beneath the robe might rise and pass through the loosely woven fabric as it was warmed, pulling in cooler air from the bottom of the robe. Either explanation would be supported by our measurements of temperatures in the air space between the robes and the skin. The temperature of the air space beneath the inner robe was approximately the same as the temperature of the surrounding air when the outer robe was black or white, despite as much as a 6 °C difference in surface temperature of the different coloured robes (47 °C for the black and 41 °C for the white) (Fig. 1).

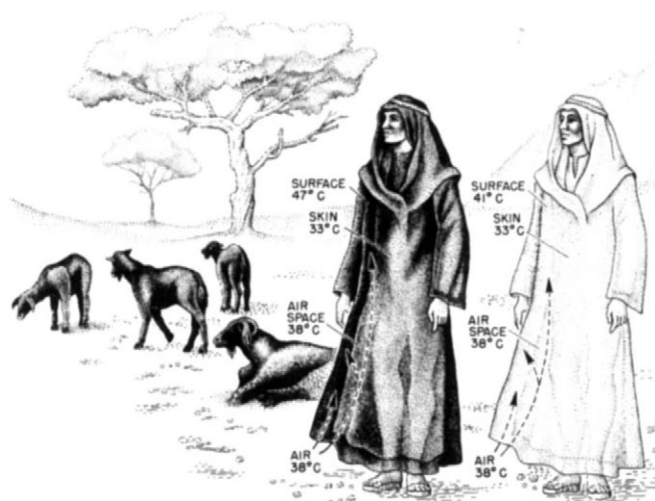


Fig. 1 Black Bedouin robes gain two to three times as much heat by radiation from the Sun as white robes, but enhanced convection of air beneath the robe carries this heat away before it reaches the skin. The temperature of the air space between the robe and the skin was the same for black and white robes and equalled the temperature of the surrounding air. The greater convection beneath the black robe might make it feel more comfortable. Mean temperatures of the robe surface, skin and air space (see text) are typical of temperatures observed when air temperature was 38 °C at mid-day, the sky was cloudless, and the subject was orientated facing the Sun.

Whether a bellows action, a chimney effect or some other mechanism explains the enhancement of convection beneath the black robes, it seems clear that the difference in radiation exchange at the outer surface of the robes is not affecting the heat exchange of the subject. Other authors have also observed that the colour of clothing has little effect on heat gain of a subject exposed to direct solar radiation^{8,9}.

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Data from kinships of monozygotic twins indicate maternal effects on verbal intelligence

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Familial resemblance in intellectual skills is well documented, but its interpretation is a source of continuing controversy. The critical problem is that a family's shared genes are confounded with its shared experiences, and controls possible in animal research (selective mating, cross-fostering, and uniform or randomised environments) do not directly apply to human subjects. Conventional twin and family methods reveal substantial genetic variance in intelligence quotient (IQ) test scores, but the same methods also document significant environmental influences. Research designs which can identify the nature of these environmental factors may effect progress in the 'IQ debate' (ref. 1). The families of monozygotic (MZ) twins provide a new research design² which permits a unique assessment of maternal influences in quantitative traits. We describe here initial applications of the design to verbal IQ, with results suggesting that maternal effects significantly contribute to familial similarity in verbal intelligence.

Maternal effects influence cognitive development in early childhood³. Observations of mothers of adopted infants, which eliminate the confounding of genetic transmission with maternal attributes, have identified specific maternal behaviour which fosters intellectual development in infancy^{4,5}. Characteristics of maternal care and qualities of the home environment during early life predict pre-adolescent IQ⁶, and longitudinal studies in London⁷ and Kauai⁸ document effects of the quality and intensity of early maternal stimulation. These and other data⁹⁻¹¹ suggest that a mother's sensitivity to her infant's needs facilitates early intellectual growth. In part, cognitive developmental differences in childhood may reflect differences in children's interactions with their mothers.

Mothers are not merely models for imitation. They establish the conditions and incentives for children's early achievement. This pervasive, but indirect influence may significantly affect a child's cognitive growth without increasing mother-child resemblance, because the relevant attributes of a mother's child-rearing may be attitudes and expectations which are uncorrelated with her IQ. Thus, the fact¹² that children's IQs exhibit no greater resemblance to their mothers than to their fathers does not preclude significant maternal effects, as such effects can¹³ and do¹⁴ occur in the absence of increased mother-child covariance.

A direct test of maternal effects on intelligence is possible within the kinships of adult identical twins. Because their twin parents are monozygotes, the children of identical twins are related to one another as conventional half-siblings; socially, they are cousins reared in different households. Because they derive from twin parents, MZ half-siblings have the same expected age and size, in contrast to conventional half-siblings resulting from death, divorce or illegitimacy. The offspring of female MZ twins form a unique group of maternal half-siblings who allow a direct measure of maternal effects on human traits. When such effects are present, the offspring of female twins will resemble one another more than do the offspring of male twins.

Table 1 Analysis of variance of offspring data from two Wechsler verbal scales

Mean squares	Information		Vocabulary	
Among paternal (MS_{Ap})	8.56	$F' = 1.56, P < 0.05$	11.12	$F' = 1.37, P < 0.10$
Among maternal (MS_{Am})	13.13		16.03	
Between paternal (MS_{Bp})	5.53		5.69	
Between maternal (MS_{Bm})	3.66		4.77	
Within paternal	3.64	NS	3.91	NS
Within maternal	4.01		3.80	
Correlations				
Paternal half-siblings	0.125	$P < 0.05$	0.200	$P < 0.10$
Maternal half-siblings	0.334		0.328	

For the Information subtest, the analysis is based on 335 offspring in kinships of 71 MZ twin parents ($N_p = 34, N_m = 37$). The analysis of Vocabulary data is based on 397 offspring in 79 MZ kinships ($N_p = 37, N_m = 42$). Because the number of offspring within each sibship varies, coefficients of the between, within and among components of variation are calculated from observed distributions of family size^{13,14}. The three coefficients do not differ for paternal and maternal families in the present data, permitting a direct evaluation of maternal influences by an F' test

$$F' = \frac{MS_{Am} + MS_{Bp}/MS_{Bm} + MS_{Ap}}{(MS_{Am})^2/N_m - 1 + (MS_{Bp})^2/N_p}$$

with d.f. = for numerator and d.f. = $\frac{(MS_{Bm} + MS_{Ap})^2}{(MS_{Bm})^2/N_m + (MS_{Ap})^2/N_p - 1}$ for denominator

In controlled matings of laboratory animals, each of a group of sires is mated to multiple dams, and the resulting litters of half- and full-sibling progeny permit a direct evaluation of maternal effects through a nested analysis of variance¹⁵. Figure 1 shows that the offspring of identical twins provide a human parallel to these conventional animal methods. Variation among the offspring of MZ twin parents may be partitioned by a nested analysis of variance¹⁶ into components arising (1) among families, (2) between sibships within families, and (3) within sibships. Because the offspring of male twins are born to genetically unrelated mothers, maternal effects, when present, will inflate the variation arising between sibships within paternal families (MS_{Bp}). Conversely, maternal effects will inflate the variance arising among half-sibships in maternal families (MS_{Am}). Finally, because all offspring within each sibship are born to the same mother, maternal effects will not affect variation arising within sibships. Accordingly, a direct test of maternal effects can be made from the partitioned variance of offspring data.

To evaluate maternal effects in intellectual development, we administered the vocabulary and information subtests from the Wechsler intelligence scales to offspring of MZ twins. Of the 11 Wechsler subtests, Vocabulary and information are the most reliable, and factor analyses of the Wechsler scales suggest that Information and Vocabulary are consistently the best predictors of general intellectual functioning over the entire age range. In addition, because the focus of this report is on maternal effects, these two verbal scales are of special interest given evidence of the mother's dominant role in a child's early development of verbal skill¹⁷.

Subjects were tested individually by trained examiners. Offspring of ages 4.0–5.11 yr were administered the Wechsler pre-school and primary scales; those aged 6.0–15.11 yr, the 1975 revision of the Intelligence Scales for Children, and those over age 16 yr were administered the Wechsler adult intelligence scale. For time intervals up to 1 yr, the inter-test correlations for vocabulary and information are as high as their test-retest stability on the individual Wechsler scales^{18,19}. Some self-selection, expected in voluntary research, is evident in our study population and verbal scores of the sample reveal elevated means and restricted variances compared with national norms. However, means and variances do not differ between twins and non-twins, males and females, or children and adults; the family providers in maternal and paternal families are closely matched in educational attainment and social-class position and comparisons between their offspring are not invalid.

Results of the analysis of variance of offspring data are presented in Table 1. The analysis provides direct evidence of a

maternal effect on verbal intelligence. For the information subtest, the intra-class correlation of maternal half-siblings, offspring of MZ twin mothers, significantly exceeds that of paternal half-siblings; comparison of the mean squares yields $F' = 1.56_{61,56}$, which enables us to reject ($P < 0.05$) the null hypothesis of no maternal influence. Results for the vocabulary subtest are similar, although the F' test does not achieve statistical significance in this preliminary sample.

The maternal effect suggested in these results could arise in any of several ways, some unique to kinships of twin mothers, some not. (1) Twin sisters may spend more time together during childhood than do twin brothers, and as a consequence of more similar childhood experience, may be more alike in their tested intelligence. We evaluated that possibility by comparing the IQ correlations of our male and female twin parents. No difference was found. (2) Female twins may maintain more frequent social contact in post-marital life and create more overlap in the IQ-relevant experience of their children. An adequate test of this possibility awaits collection of self-reported frequency of social interaction within kinships, but a crude test was made by comparing the geographical propinquity of maternal and paternal half-sibships. Again, no difference was found. (3) Assortative mating for IQ by MZ twin sisters may exceed that by MZ twin brothers. Asymmetric assortative mating was directly evaluated by comparing the resemblance of wives of twin brothers to husbands of twin sisters. The spousal correlations do not differ. (4) Identical twin sisters may provide more similar patterns of child-rearing than do the unrelated wives of twin brothers, such that the greater resemblance of their children reflects greater resemblance of relevant maternal behaviour. (5) The greater resemblance of maternal half-siblings may reflect

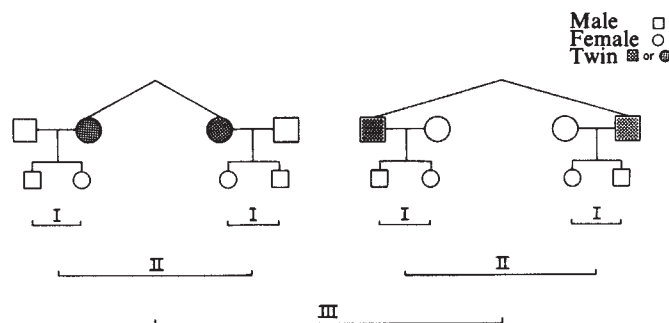


Fig. 1 Variation in offspring data in kinships of MZ twin parents. I, A component of variation arising within sibships; II, variation arising between sibships nested within families; III, variation arising among families.

the reduced variability of intra-uterine environment with which identical twin mothers provide their children. The last two possibilities reflect pre- and postnatal maternal influences not limited to kinships of twin parents, and, although we have no direct tests of them, research with mothers of singletons suggests their plausibility.

Maternal effects modulate the expression of familial mental retardation²⁰, and the data presented here, obtained from normal children reared by their biological parents in typical home environments, provide direct evidence of significant maternal influences on variation in tested IQ. Indeed, the correlation of maternal half-siblings approaches that typically reported for full siblings! We remain uncertain as to whether the source of these maternal influences is pre- or postnatal, but we emphasise that they are found in the context of significant genetic variance²¹ to clarify a widespread confusion—that demonstrations of genetic variance in IQ test scores nullify social efforts to enrich early intellectual development. Heritability of IQ does not negate its social modifiability. Evidence of significant maternal effects may serve to redirect the polemical IQ debate towards constructive efforts to identify environmental factors in cognitive development.

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Distracting information, motor performance and sex differences

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It is often argued that, in general, men are superior to women in perceptual motor skills (for example, car driving). Extensive reviews of the relevant literature do not give convincing evidence of sex differences in manual dexterity¹ or motor skills in general². However, in the studies reported here, variables have been isolated which demonstrate that sex differences do exist in two perceptual motor tasks. These studies showed that females are adversely affected by irrelevant stimuli while performing tasks permitting freedom in movements but perform as well as males when the movement is stereotyped.

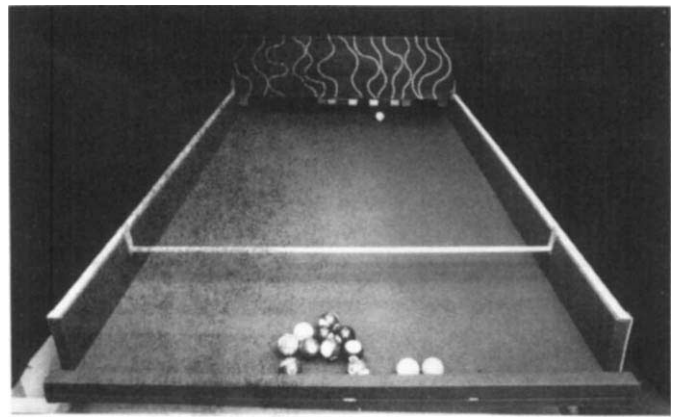


Fig. 1 The experimental set-up. The subject was to roll the ball at the target aiming at the centre flap. In Ward-games *a* and *b* the target comprised seven flaps—the centre flap scored 4; in Ward-game *c* nine flaps were used, the centre flaps scored 5. The landmarks shown were used in Ward-game *c*. In the no-landmark condition the lines were absent.

We first established the performance variables that have direct relevance to motor programming in an aiming task, without considering sex differences. The task was carried out on a Ward table 213 × 114.5 cm (see Fig. 1). At one end was a target consisting of seven 5-cm-wide flaps, either stationary in the middle of the table, or moving from side to side at 20 excursions per min. Subjects had to hit the target with a billiard ball. The score depended on the accuracy of the hit. Target missed scored 0; the outer two flaps scored 1 each; the middle flap scored 4, with increasing scores for the second and third flaps. Several variables were tested in a between-subjects, randomised block design, but for the present purposes, only motor programming demands and sensory information are considered. Motor programming demands varied from simple stereotyped to complex movements, involving temporal and/or spatial organisation. (1) The subject was required to 'shoot' the ball out of a spring-loaded 'gun' at the moving target. The subject had to make the temporal decision when to press the button to release the ball. (2) The subject rolled the ball manually at the stationary target, requiring a directional decision only. (3) The ball was rolled manually at the moving target, necessitating both spatial and temporal programming.

Sensory information was varied by the presence or absence of landmarks. Above the target was a matt blackboard in the no-landmark condition, and four regularly placed vertical white lines were displayed on the blackboard in the landmark

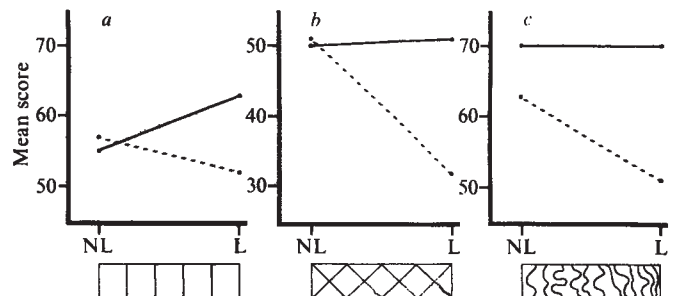


Fig. 2 Results of three Ward-games with the ball manually rolled at the moving target. Maximum score for: Ward-game *a* [condition (3)], 30 trials = 120; Ward-game *b*, 25 trials = 100; Ward-game *c*, 25 trials = 125. Males: —; females: ---. L, landmark; NL, no landmark. The patterns below each graph denote the visual stimuli used as landmarks.

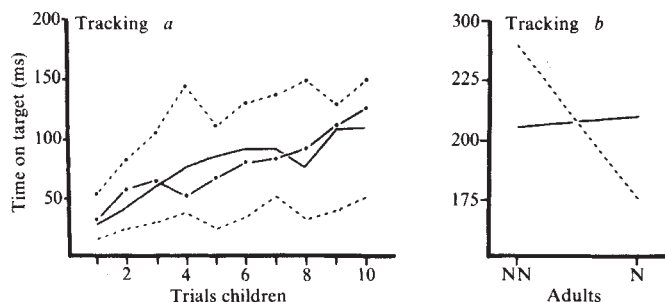


Fig. 3 Results of tracking studies in adults and children. Males: —; females: ---. Tracking *a*, noise: no dots; no noise: dots marking data points. Tracking *b*, N: traffic noise through ear-phones; NN: no noise.

condition. These landmarks were expected to aid in the timing of the movement.

The experiment was conducted as the 'Ward-game' championship and we noticed that males dominated the finals. The results showed that in none of the movement conditions did the landmarks assist male performance, whereas females performed significantly worse with landmarks than without landmarks in conditions (2) and (3) [(2) $t(32) = 3.52$, and (3) 3.92 , $P < 0.01$], although in (1) no significant difference was found. Thus, landmarks affected females only, and were irrelevant for the performance of males (Fig. 2).

We carried out two further experiments with the subjects manually rolling the ball at the moving target [condition (3) Ward-game *a*], but varied the visual patterns (Fig. 2). The results confirmed the findings of Ward-game *a*. [Ward-game *b*: landmark main effect $F(1, 36) 5.102$, $P < 0.03$; sex main effect $F(1, 36) 7.662$, $P < 0.01$; interaction 9.072 , $P < 0.005$; Ward-game *c*: with planned comparison: males versus females with landmark $F(1, 36) 9.84$, $P < 0.01$; males versus females without landmarks $F(1, 36) 0.28$, not significant.]

To establish that sex differences were not task specific and relevant to visual stimuli only, two pursuit tracking studies were run. In these, a triangular track (33 r.p.m.) was used, with recorded traffic noise as the irrelevant stimulus. For the control groups the noise was omitted. In the tracking *a* study, the subjects were 7–12-yr-old children, and in tracking *b*, adults were used. The Ward-game results were confirmed [tracking *a*: auditory main effect $F(1, 28) 6.96$, $P < 0.01$; sex main effect not significant; interaction 5.81 , $P < 0.01$; tracking *b*: ANOVA results not significant, t -test on female performance with versus without auditory noise: $t(22) = 4.214$, $P < 0.001$] (see Fig. 3).

In a further three experiments with stereotyped aiming movements³ and visual distractors, no sex difference was found.

Thus, it seems that, as a group, females from the age of 7 yr are adversely affected by irrelevant stimuli in the performance of motor skills in which spatial freedom of movement programming is possible. It can be argued that the input which is processed preceding and determining the motor programme⁴ is over-inclusive in females, but selective in males.

The implications of these findings are: (1) contradictory results in the literature on sex differences in motor skill may be due to stimulus configuration in the experimental situation and/or to motor programming demands of the task; (2) most females might be adversely affected by distracting stimuli when operating complex machinery and in car driving; (3) industrial training and driver education may need to be re-examined.

This research was supported by the University of Western Australia Research Grants Committee. We thank Judith Cullity and Jocelyn Brown for their assistance in running some of the experiments, and Peter Bourn, Michael Caracciolo, Chris Edwards, Claire Fletcher, Mike Jones, Rhonda King, Shelagh Morgan, Hanja Sambrailo and Keith Wise, who acted as experimenters in some of the studies.

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Origin of the mammal-like reptiles

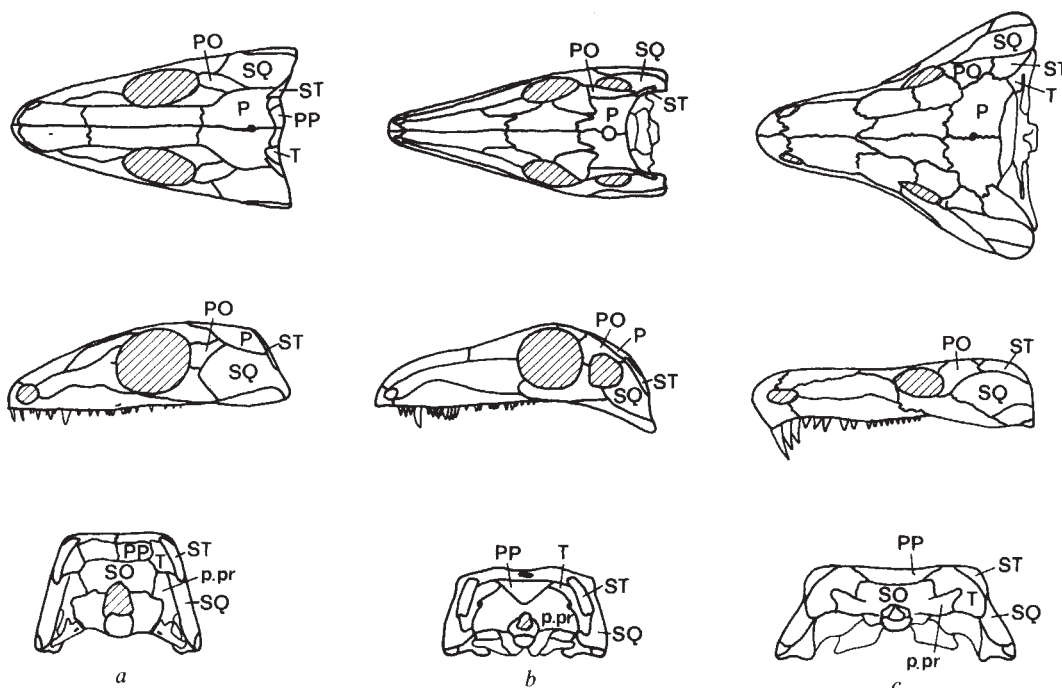
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The first major adaptive radiation of reptiles consisted of the Synapsida, or mammal-like reptiles, which are now totally extinct, but which dominated the terrestrial fauna from the late Pennsylvanian, throughout the Permian and for much of the Triassic periods. The mammals arose from advanced synapsids in the Upper Triassic, at which time the dinosaur reptiles were rapidly replacing the mammal-like reptiles as the principal terrestrial forms. The orthodox view^{1,2} of the ancestry of this important stage in vertebrate history is that the synapsids arose from a hypothetical stem-reptile of the family Romeriidae, the group closest to the ancestry of all the other reptile lineages. The principal difference between romeriids and the primitive synapsids is the development of a space, or temporal fenestra in the cheek region of the skull of the latter. However, the structure of this part of the skull of the romeriids cannot satisfactorily be considered ancestral to the synapsid temporal region on either comparative anatomical or functional grounds. As I propose here, the kind of skull which would be expected to evolve into that of a synapsid is the type possessed by the archaic 'quasi-reptile' *Limnoscelus* and its possible relative *Romeriscus*.

The typical synapsid temporal fenestra is already fully developed in the earliest known skull³, that of the pelycosaur *Archaeothyris* from deposits of Westphalian D age, in the Pennsylvanian of Nova Scotia (Fig. 1b). The fenestra is bounded above by the postorbital and squamosal bones, and the parietal bone is not in contact with the squamosal. Another point to note is that the supratemporal bone, although small, still contacts the postorbital. In contrast, the romeriid skull (Fig. 1a) has the postorbital restricted anteriorly, so that there is a long line of contact between the parietal and squamosal, while the supra-temporal is reduced to a small, superficial bone right at the back of the skull. The only explanation offered⁴ for the difference in bone configuration is that when the temporal fenestra formed in a romeriid-like skull, there was a secondary extension of the postorbital bone backwards, above the new fenestra, to strengthen this part of the skull. It is not clear, however, why the temporal region of the skull should be strengthened in this way. If fenestration of a romeriid skull were to occur, then the space would inevitably develop between the parietal bone above and the squamosal bone below, yet these are two of the largest, most robust bones of the skull, and their mutual contact both in front of and behind the supposed new fenestra would have provided adequate strength. Indeed, this is probably what happened in certain other reptile lines where the fenestra is bounded dorsally by the parietal and squamosal. An alternative view of the origin of the synapsid skull is that the ancestral condition of the temporal region consisted of a large supratemporal bone which was in contact anteriorly with the postorbital. This condition is found in the skull of the limnoscelids, which are early reptile-like forms best known as *Limnoscelus* itself⁵. *Limnoscelus* occurs in the Lower Permian of North America and is a relatively large animal. The skull is unique among early reptiles, for the supra-temporal bone is very large and extends far enough forwards to make a broad contact with the postorbital (Fig. 1c). The supposed remnant⁶ of the old crossopterygian fish hinge-line

Fig. 1 *a*, Skull of the primitive romeriid *Paleothyris* in dorsal (top), lateral (centre) and posterior (bottom) views. Note the extensive contact between the parietal (P) and squamosal (SQ), the short postorbital (PO) and small supratemporal (ST). No temporal fenestra is present (after Carroll and Baird). *b*, Skull of pelycosaur synapsids, *Archaeothyris* in dorsal view (top) and lateral view (centre); *Varanops* in posterior view (bottom). Note the contact between the postorbital (PO) and supratemporal (ST) and postorbital and squamosal (SQ) above the temporal fenestra (after Romer and Price, and Reisz). *c*, Skull of the primitive reptile-like *Limnoscelis* in dorsal (top), lateral (centre) and posterior (bottom) views.



Note the large contact between the postorbital (PO) and supratemporal (ST) bones above the line of weakness between these two bones and the squamosal (SQ) (after Romer). m, adductor mandibuli muscle; P, parietal; PO, postorbital; PP, postparietal; p.pr, paroccipital process; SO, supraoccipital; SQ, squamosal; ST, supratemporal; T, tabular; t.ap, temporal aponeurosis.

persists in the form of a failure of the squamosal to unite firmly with the supratemporal and postorbital above. Thus the cheek and skull table must have been held together by soft connective tissue, probably to allow a slight intracranial kinesis at this point. Another, much earlier form *Romeriscus* has been described as a limnoscelid by Baird and Carroll⁷ (Fig. 2a), on the basis of a similar configuration of the temporal region. In fact, the skull is very incomplete and badly crushed, but certainly the supratemporal is large, and extends anteriorly sufficiently for it to be presumed to meet the missing postorbital. And there is no apparent suture between the supratemporal and squamosal, indicating the presence of a line of weakness. Whether *Romeriscus* is a limnoscelid or not is doubtful⁶, but it does suggest that the basically limnoscelid type of temporal region of the skull existed early, for it dates from the Westphalian A, earlier than any of the true reptiles.

The arrangement of the skull bones of *Limnoscelis* offers a good model for the ancestral state leading to the fenestrated synapsid condition. The classic view that a temporal fenestra is a window out of which the contracting jaw muscles can bulge has been discredited by the observation that in modern fenestrated reptiles the muscles do not so bulge, and that the muscle fibres tend to attach to the edges of the fenestra and to connective tissue covering it. A more acceptable view is that of Frazzetta⁸, who suggested that the role of the fenestra is to provide bony edges, to which the muscles can be more strongly anchored. In the line leading to pelycosaurs, an increase in body size appears to have occurred, for all the early forms are larger than other contemporary reptiles³. Therefore a relative increase in the mass of the adductor jaw musculature would be expected. If the ancestral, unfenestrated skull was similar to that of limnoscelids, some of the muscle fibres would tend to become attached to the connective tissue between the skull table and cheek (Fig. 2b, top). In effect, these particular fibres would be attached to the lateral edge of the postorbital and supratemporal bones, giving them a stronger anchorage than other fibres which merely attached to the internal surfaces of the skull bones. An increase in the area of the connective tissue between cheek and table so that increasing numbers of muscle fibres could attach to it would be expected, leading to the development of a gap, or fenestra,

between the postorbital and supratemporal above and the squamosal below (Fig. 2b, bottom), covered over by an aponeurotic sheet. The weakest point of the temporal region would have been the supratemporal bone, for it is small and not strongly attached posteriorly. Expansion of the postorbital posteriorly, below the supratemporal, to meet and brace against the squamosal followed, thereby strengthening the post-fenestral region of the skull, and giving rise to the characteristic form of the synapsid postorbital bone.

Two further arguments support this hypothesis of the origin of the synapsid skull. The first concerns the occipital structure of the skull. In the romeriids, the tabular bone is small and restricted to the dorsal part of the occiput, and therefore makes at best a very limited contact with the paroccipital process (Fig. 1a, bottom). In contrast, the pelycosaurs possess a large tabular which extends ventrally to make a massive, bracing contact with the paroccipital process (Fig. 1b, bottom). Panchen noted this point⁶, and concluded that the common ancestor of romeriids and pelycosaurs was more primitive than any known form. In fact, the limnoscelid tabular and paroccipital process have a relationship very similar to that of the pelycosaurs (Fig. 1c, bottom). Functionally, retention of the tabular-paroccipital process brace in pelycosaurs would be expected, as a further manifestation of the need for strengthening devices in the potentially weak post-fenestral region of the skull. The second argument is that in certain generally primitive pelycosaurs such as *Eothyris*¹ (Fig. 2c) and *Oedaleops*⁹ the supratemporal is a large bone, making extensive contact with the postorbital directly above the temporal fenestra. It is not unreasonable therefore to suggest that a large supratemporal bone, quite unlike that of the romeriids, is actually the primitive synapsid condition.

The conclusion is, therefore, that the synapsid skull evolved from a limnoscelid-like skull rather than from that of a romeriid, and that the temporal fenestra of the mammal-like reptiles evolved directly from the remnant of the crossopterygian hinge line. This is not to say, of course, that taxonomically the limnoscelids are the ancestors of the synapsids, merely that the limnoscelids have retained the ancestral configuration of the temporal region of the skull. It has long been supposed that a

fundamental reptilian feature is the parietal lappet, which is the lateral process of that bone which contacts the squamosal and replaces topologically the intertemporal bone of early labyrinthodont amphibians. This arrangement contrasts with those labyrinthodonts which themselves lost the intertemporal, for here the area occupied by that bone is taken over by the

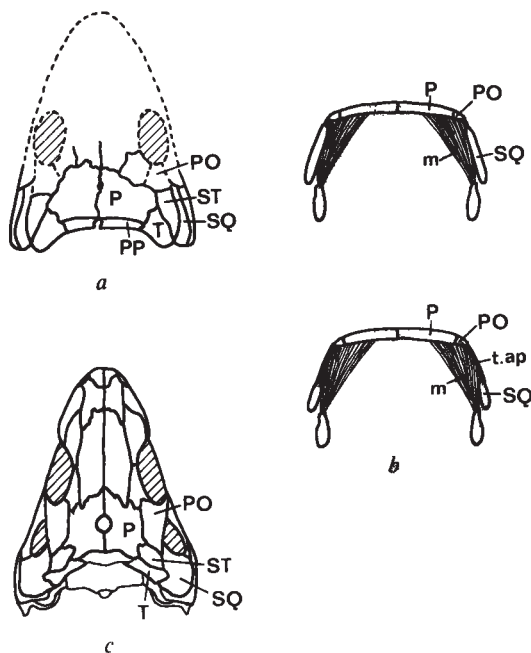


Fig. 2 *a*, Skull of the possible early limnoscelid *Romeriscus* in dorsal view (after Baird and Carroll). *b*, Above, diagrammatic cross-section through the temporal region of the skull of a limnoscelid-like form, in which the adductor jaw muscles (*m*) are beginning to gain an attachment to the connective tissue binding the postorbital (*PO*) of the skull roof to the squamosal (*SQ*) of the cheek. Below, further development of this trend, with enlargement of the gap to form a temporal fenestra, covered by a large connective tissue aponeurotic sheet (*t.ap*), leading to the synapsid skull pattern. *c*, Dorsal view of the skull of a very primitive pelycosaur synapsid *Eothyris*. Note the retention of a large supratemporal in contact with the postorbital (after Romer and Price).

postorbital and supratemporal. On the argument presented here, the synapsid line at least never possessed a parietal lappet. Whether the parietal lappet of the romeriids and other primitive reptiles does indeed indicate a separate loss of the intertemporal, or whether it reflects a subsequent reduction of the postorbital and supratemporal bones is not known. The latter seems the more probable in view of the many points of similarity that do exist between limnoscelids, romeriids and pelycosaurs.

Juvenile ceratopsians from Mongolia—the smallest known dinosaur specimens

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Among dinosaurs collected by the Third Asiatic Expedition of the American Museum of Natural History (New York) are two juveniles each of which is smaller than any hitherto reported dinosaur specimen. Their overall anatomy resembles that of the type specimen of *Psittacosaurus mongoliensis* (AMNH 6254)¹, which was collected from the same horizon and locality: the Lower Cretaceous Oshih Formation, Artsa Bogdo Basin, Mongolian Peoples' Republic. The anatomy of the new specimens confirms *Psittacosaurus* as a ceratopsian, not an ornithomimid, and their minute size precludes both post-hatching parental care and endothermy.

Of the two specimens, AMNH 6535 includes a skull and partial mandibles and AMNH 6536 includes a skull, mandibles, partial pes in articulation and numerous postcranial elements (Fig. 1). Important cranial features include: skull triangular in dorsal view; short deep snout with parrot-like beak; small nostrils far dorsal; massive jugal with small ventrolateral flange

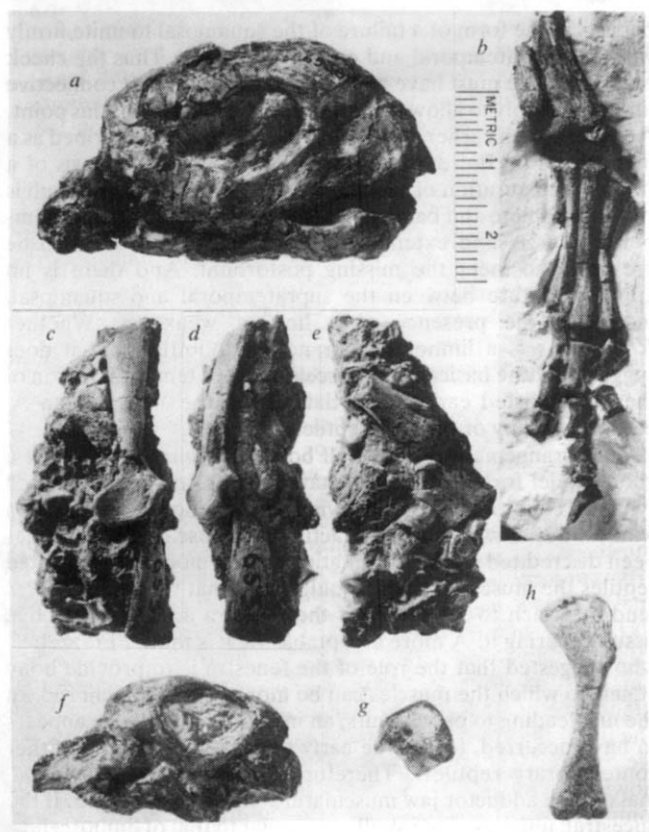


Fig. 1 Representative material of the two smallest known dinosaur specimens. *a-e, g* and *h*, AMNH 6536; *f*, AMNH 6535. *a*, Skull and mandibles. *b*, Left pes with distal ends of tibia and fibula, astragalus, calcaneum, metatarsals II, III, IV, and partial metatarsal V, and a partial set of phalanges. *c, d*, and *e*, three views of a block of bone containing two scapulae, partial ilium, partial ischium, proximal end of a femur, several vertebrae, and sacral ribs. *f*, Skull and partial mandible. *g*, Right coracoid. *h*, Left humerus. Scale divisions in mm (shown approximately natural size).

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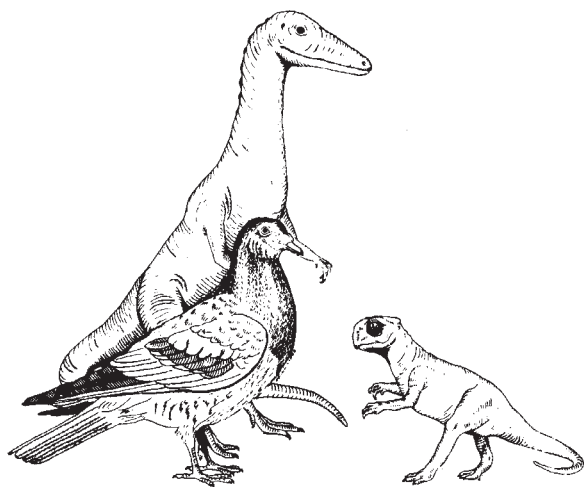


Fig. 2 Restoration of the smallest known dinosaur, a juvenile *Psittacosaurus mongoliensis*, based on AMNH 6535. Included for size comparison (on the left) are *Compsognathus longipes* and a common pigeon (*Columba livia*). Illustration by Matthew Hyman.

or spike; and massive curved mandible with triangular coronoid process. A rostral bone, present on both juvenile skulls and the type specimen of *Psittacosaurus mongoliensis*, is a synapomorphic character diagnostic of the suborder Ceratopsia. Wear on the teeth of both juvenile skulls indicates a diet of abrasive vegetation, and suggests that a newly hatched *Psittacosaurus* would have been smaller than either of these specimens. Pedal conformation, humeral shape, elongate tapering scapulae and femora with cylindrical heads and curved shafts (Fig. 1) all indicate a gracile, tridactyl biped. That these specimens are juvenile is indicated by the enormous orbits, relatively large braincase, arched cranial roof, short snout and neural arches not fused to centra. Using basicranial length for comparison, the smallest known dinosaur specimens include the following: *Lesothosaurus diagnosticus*, 94 mm; two juvenile *Protoceratops andrewsi*, 76 mm and 62 mm; *Compsognathus longipes*, 70–75 mm, and *Bagaceratops rozhdesvenskyi*, 47 mm (refs. 2–6). The juvenile *Psittacosaurus* skulls measure 42 mm (AMNH 6536) and 28 mm (AMNH 6535). Estimated total body length, snout to tail tip, obtained by scaling down the complete skeleton of *P. mongoliensis* (AMNH 6254) is 395 mm for AMNH 6536, and 265 mm for AMNH 6535. These values make no allowance for allometric changes in proportions, and insofar as the skull is relatively larger in juveniles, the actual total length for AMNH 6535 is probably between 230 and 250 mm. The latter juvenile was smaller than a common pigeon (*Columba livia*) and less than one-third the length of *Compsognathus longipes* (750–810 mm)⁶, also a juvenile specimen and often cited as the smallest known dinosaur (Fig. 2).

Dinosaur egg morphology indicates a pattern of nest construction similar to that of crocodilians and megapodes (chicken-like, terrestrial birds of Australasia)⁷, and parental attendance of such nests is reasonably inferred for dinosaurs. The usual thickness of dinosaur eggs may have reduced breakage by attendant parents of large size and fully upright stance. However, the minute size of these juvenile *Psittacosaurus* compared with the adult (body mass of juvenile about 0.7% of adult mass) makes post-hatching care by parents virtually impossible, both because the parent could not effectively feed so tiny a juvenile, and because the young would be liable to crushing at the least misstep of the adult. Tooth wear and well-ossified limb bones point to precocial self-feeding by hatchling *Psittacosaurus*. The surface area to body mass ratio of these juveniles would promote heat loss at a much higher rate than in adults. Exceptionally small neonates of modern endotherms are commonly ectothermic at birth, with endothermic physiology developing together with a more favourable surface

area to body mass relationship^{8,9}. Egg volumes of *Protoceratops* and *Hypselosaurus* infer a hatchling to adult size discrepancy even greater than that of *Psittacosaurus*¹⁰. Regardless of adult physiology, it is unlikely that such relatively tiny juvenile dinosaurs were endothermic in a mammalian-avian sense.

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Male and female visual neurones in dipterous insects

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In many insects some visually guided behaviour patterns differ between the sexes. For example, male hoverflies and houseflies chase females in the air using visual cues but are not chased by females^{1–3}. Presumably information processing by nerve cells also differs. The genetic and histochemical identification of a brain area in *Drosophila* that must be male for male behaviour to occur⁴ suggests that circuits for sexual behaviour are localized. Recent anatomical studies of houseflies (*Musca domestica*) have demonstrated certain visual neurones present only in males⁵. I report here results from another species (the blowfly, *Calliphora erythrocephala*) demonstrating another kind of sexual difference of neural architecture in the visual system. In this fly dimorphism is expressed by differences in the shapes of analogous neurones in males and females, as well as by the presence of some cells in only one sex. The sexually dimorphic neurones of both sexes 'view' a region of the visual field coinciding with the region of binocular overlap.

The structures described here are from the lobula, the third visual neuropil. In this neuropil, columnar arrangements of small neurones precisely represent the facet organisation of the retina, and any dendritic field in the lobula can be represented as a map on the retinal surface^{5,6}. Assemblies of nerve cells in the lobula are revealed by silver intensification of cobalt ions introduced either from a pool of cobalt chloride over a cut in the thoracic ganglion or from a smaller pool diffusing from a micropipette inserted into the brain. In the first case cobalt ions pass 'trans-synaptically' from dendrites of descending neurones into presynaptic relay cells originating in the lobula (Fig. 1a, b)⁷. In the second case cobalt appears to be taken up directly by one or more populations of visual interneurons⁸. The same neuronal arrangement can be revealed reproducibly in different brains. The results reported here are obtained from examination of 80 males and 60 females.

The shapes of many neurones do not significantly differ in males and females and comprise similarly structured networks. These are not described here; instead the emphasis is on morphologies characteristic of one or the other sex.

Lobulae of male blowflies have certain prominent neurones, each with characteristic shape, dendritic domain and axonal destination. Three of these are the male lobula giants⁵ (MLG 1–3) illustrated in Figs 1 and 2. Two (MLG 1, 2) reside in the upper frontal lobula where their unistratified dendritic fields interact with relays from upper frontal ommatidia of the eye

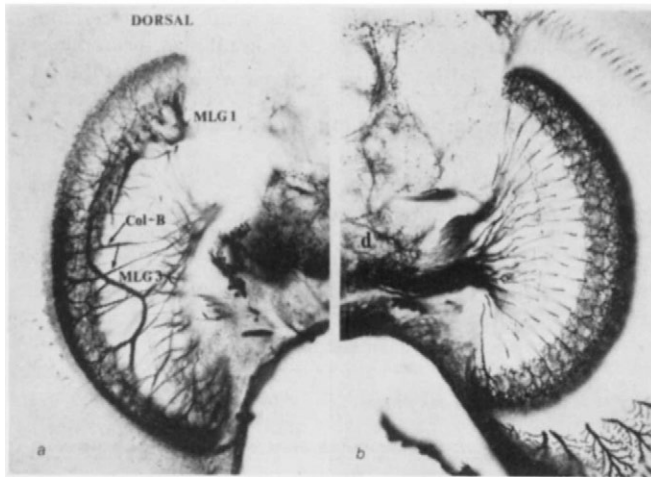


Fig. 1 Silver intensified neurons in male (a) and female (b) lobulae of *Calliphora erythrocephala*. The two pictures illustrate palisades of Col A neurones, parts of two male lobula giant neurones (MLG 1 and 3), and some Col B neurones which have taken up cobalt after its initial introduction into cut thoracic ganglia ($\times 100$).

(Fig. 3). Their cell bodies lie as a pair posteriorly above the upper margin of the lobula plate. The axon of MLG 1 passes to the rear of the contralateral mid-brain, ending among dendrites of descending neurones. The axon of MLG 2 ends at the equivalent ipsilateral region. Terminal branching patterns of MLG 1 and MLG 2 are similar. The third giant neurone (MLG 3) has a lyre-shaped profile. Its dendritic branches invade the anterior lobula along a strip that is narrow dorsally and wider ventrally (Fig. 3). The location of its cell body has not been identified. Its axon projects to the vicinity of a descending neurone in the medial ipsilateral protocerebrum.

In the female lobula there are four special neurones (female lobula giants; FLG 1-4). The dendrites of two of these (FLG 1, 2) invade the upper frontal lobula. FLG 1 is characterised by a unistratified field of spiny dendrites and, like MLG 1, its axon ends among dendrites of descending neurones in the rear of the contralateral mid-brain. The dendrites of FLG 2 are multistratified and distal to FLG 1 in the lobula. Like MLG 2, its axon passes to the rear of the ipsilateral mid-brain. The cell bodies of FLG 1 and 2 lie as a pair between the upper margin of the lobula and the upper margin of the lobula plate. A small lyre-shaped neurone (FLG 3) invades a narrow anterior strip of the lobula both above and beneath its equator. Like MLG 3, its axon projects ipsilaterally to the medial protocerebrum. FLG 4 invades a fronto-ventral strip of the lobula. Its axon projection has not been followed and the cell body locations of FLG 3 and 4 are not known.

Sexual dimorphism is also expressed among palisades of columnar neurones common to both sexes. Type A columnar cells (Col A)⁵ are distributed throughout male and female lobulae and their axons project to the same region of the medial ipsilateral protocerebrum as MLG 3 and FLG 3. Col A shapes and distributions are similar in the two sexes (Fig. 2). However, in the upper region of the male lobula, where they overlie MLG 1 and 2, Col A cells show a graded change of dendritic morphology from multistratified to bistratified. In females, Col A neurones are uniform over the whole lobula, including the region occupied by FLG 1 and 2.

Both sexes of blowfly have in the lobula an upper-frontal row of columnar neurones serving exclusively the extreme frontal retina. In males, this row consists of two cell types: Col B ventrally, and Col C dorsally. In females the row consists only of Col B cells, ventrally and dorsally (Fig. 2). Col B cells have multistratified dendrites and long oval dendritic domains. Their axons project dorsally to ipsilateral protocerebrum in both sexes. Col C cells are distinguished by flattened dendritic expansions emitting numerous thin processes. Col C axons

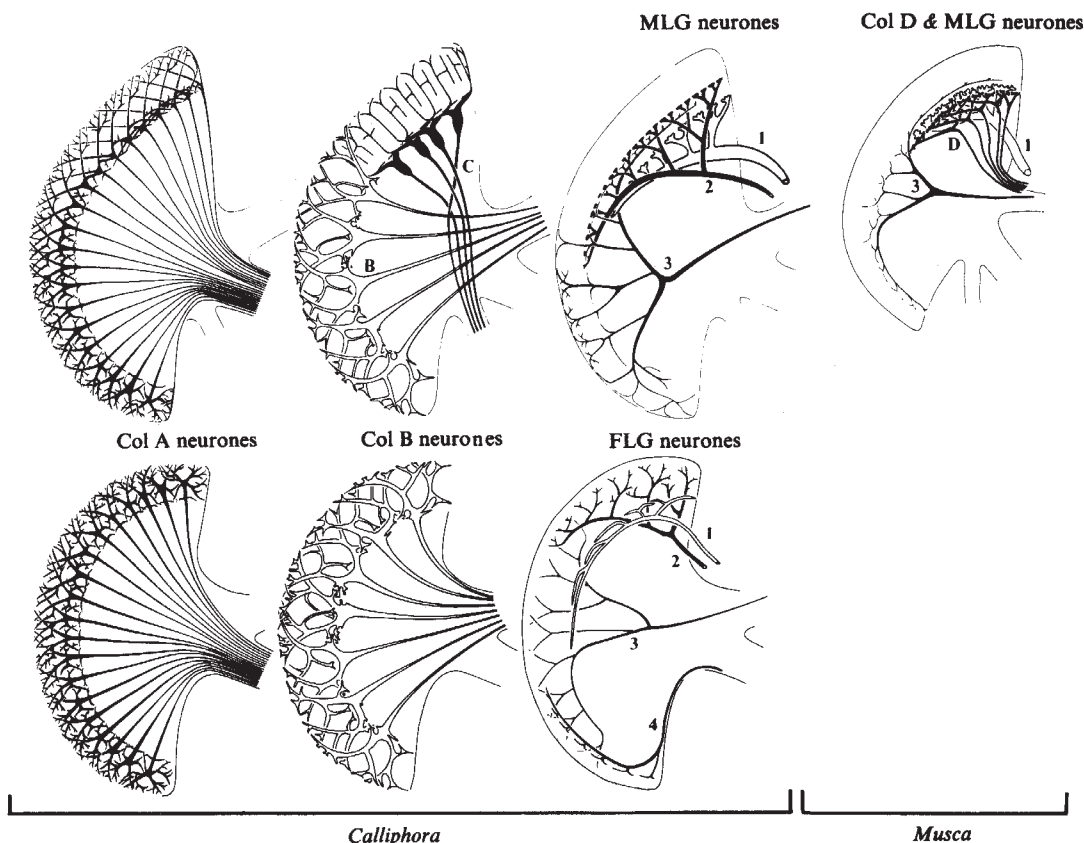


Fig. 2 Diagram of neurones in the lobula of male blowflies (upper row) and females (lower row) as seen in vertical sections of the lobula. Dorsal is to the top, with the midline to the right. Type A columnar neurones (Col A) are found throughout the lobula and are identical between the sexes except in the upper frontal zone of the male lobula. Col B neurones are found in the anterior of the lobula in both sexes. However, in males they are absent from an upper region. Col C neurones are found only in the anterior upper male lobula. Three unique male lobula giant neurones (MLG 1-3) and four unique female lobula giant neurones (FLG 1-4) are also found. Although termed female giants the diameters and processes of FLG neurones are usually much smaller than those of MLG neurones. The inset (upper right) shows the homologues of the MLG 1-3 neurones in the housefly, where a palisade of columnar cells (Col D) appears to substitute the single MLG 2 of *Calliphora* (see ref. 5).

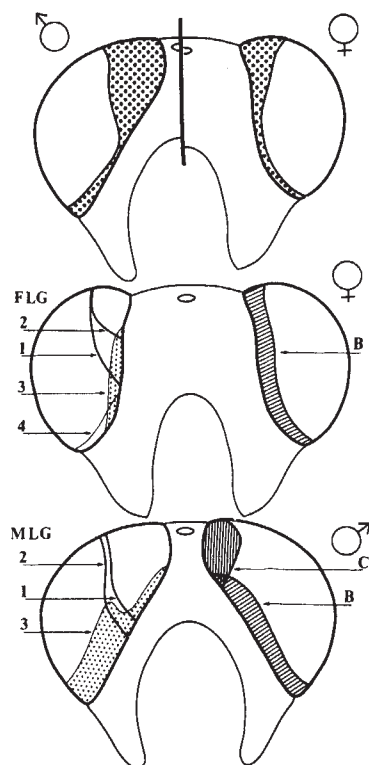


Fig. 3 Maps of dendritic fields demonstrate the sexual dimorphism. The top figure juxtaposes front views of male and female half-heads to show differences in eye position and zones of binocular overlap⁹ (indicated by heavy dots). The middle figure shows the domains of FLG neurones mapped on to the retina to the left. The domains of FLG 1 and FLG 2 are above the lines indicated; the domain of FLG 3 is dotted and the domain of FLG 4 is below the line indicated. The domain of female Col B cells is shown in hatching on the right. The lower figure illustrates the domains of MLG 1-3 and male Col B and Col C neurones. Note the general correspondence between the zones of binocular overlap and the domains of Col B, Col C, MLG and FLG neurones.

project ventrally to a median locus in ipsilateral protocerebrum.

The styles of sexual dimorphism in the blowfly and housefly show interesting differences. Neurones analogous to MLG 1, MLG 3 and Col C are found in the lobulae of male houseflies⁵. However, there is no single element corresponding to MLG 2: instead, the housefly has a small cluster of columnar cells (Col D⁵, Fig. 2) collectively covering the same domain of the lobula. Like MLG 2 of *Calliphora*, their axons project ventrally into the ipsilateral rear protocerebrum. No neurones corresponding to FLG 1-4 were found in female houseflies although they were sought with the cobalt method, with reduced silver stains, and by reconstruction from serial 3- μ m Araldite-embedded sections stained with methylene blue⁵.

Comparisons between the sexes of *Calliphora* lead to the following conclusions. Three unique neurones in the male and female are probably analogous. The location of dendrites is similar and the cell body locations of two cells (MLG 1, 2 and FLG 1, 2) are similar. Similarly shaped neurones in males and females send axons to the same regions. Comparable neurones differ with respect to morphology, axon diameter, dendritic domain and layer relationships. These differences may reflect different synaptic connections in males and females. Other neurones (FLG 4 and Col C) are present in one sex but apparently have no analogue in the other sex.

The sexual dimorphism of lobula neurones can be summarised by projections of their dendritic domains out to the retinal surface, as in Fig. 3. This figure also compares regions of binocular overlap in males and females⁹. The overlap is greatest in the upper frontal retina of the male where there is also an

extra zone of ommatidia not present in the female¹⁰⁻¹². The MLG 1 and 2 fields, and the Col C assembly, correspond to this zone (Fig. 3). In the female, FLG 1 and FLG 2 cover slightly more of the retina than the female binocularity zone. In both sexes the strip of Col B neurones corresponds to the binocular overlap zone more ventrally. In females, FLG 3 and 4 lie within this zone whereas in males the ventral part of the MLG 3 domain is significantly wider than this zone.

It has been suggested that the upper front part of the eye of male houseflies may play a significant role in visually guided sexual behaviour³. Are the sexually dimorphic neurones important in sexually dimorphic behaviour? Their large size should permit exploration of this question by electrophysiological methods.

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Feeding behaviour of tsetse flies infected with salivarian trypanosomes

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Although much is known about factors which determine infection rates of salivarian trypanosomes (subgenera *Nannomonas*, *Duttonella* and *Trypanozoon*) in the tsetse fly *Glossina*^{1,2}, it is not clear why infection rates of *Trypanozoon* are high in mammalian hosts but low in wild-caught *Glossina*^{3,4} and why trypanosomiasis occurs where *Glossina* is not readily detectable. We report here that the feeding behaviour of trypanosome-infected *Glossina* differed from that of uninfected control flies. Infected flies probed more frequently and fed more voraciously. We describe a specific relationship between trypanosomes and the mechanoreceptors responsible for detecting the rate of blood flow⁵⁻⁷, and show how infection affects that rate in the labrum. We suggest that the observed differences in feeding behaviour result from impaired function of the labral mechanoreceptors in infected *Glossina*.

Glossina m. morsitans and *Glossina austeni* were obtained as pupae. Teneral *G. m. morsitans* were fed on mice (ICR) infected with *Trypanosoma (Trypanozoon) brucei* subsp. (ST1B 247) isolated in Serengeti, Tanzania, from *Alcelaphus buselaphus* (hartebeest) in 1971 and cryopreserved after one passage in the rat. Control flies received an uninfected blood meal at the same time and all flies were subsequently maintained as described previously⁸. Infected flies were detected by inducing individuals to probe on warmed slides 14 days after the infective feed and then feeding them on mice to verify their infectivity.

Forty infected and forty uninfected flies (20 males and 20 females in each group) were placed individually in glass tubes

Table 1 Probing and feeding patterns of infected and uninfected *Glossina m. morsitans*

State of flies	Starving for 24 h			Starving for 48 h		
	No. feeding	No. feeding on first probe	Mean no. of probes $\pm$ s.e. before feeding	No. feeding	No. feeding on first probe	Mean no. of probes $\pm$ s.e. before feeding
40 Infected flies	40	3	5.08 $\pm$ 0.40	40	4	4.68 $\pm$ 0.37
40 Uninfected flies	28	13	1.89 $\pm$ 0.21	38	24	1.53 $\pm$ 0.13

The flies were 20–35 days old.

Table 2 Factors by which the required pressure drop $-\Delta\bar{P}$ would need to be increased for a given proportion of length colonised by trypanosomes $m(L)$ and for a given proportionate reduction in diameter $n(D)$ as a result of trypanosome infection if the same flow rate in the labrum was to be maintained

		Proportion of length infected										
		$m(L)$	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Proportion of diameter unoccluded	$n(D)$											
	1.0	—	—	—	—	—	—	—	—	—	—	1
	0.9	1.052	1.10	1.16	1.21	1.26	1.31	1.37	1.42	1.47	1.52	
	0.8	1.14	1.29	1.43	1.58	1.72	1.86	2.01	2.15	2.30	2.44	
	0.7	1.31	1.63	1.95	2.27	2.58	2.90	3.22	3.53	3.85	4.16	
	0.6	1.67	2.34	3.01	3.69	4.36	5.03	5.70	6.37	7.04	7.72	
	0.5	2.5	4	5.5	7	8.5	10	11.5	13	14.5	16	
	0.4	4.81	8.61	12.42	16.23	20.03	23.84	27.64	31.45	35.26	39.06	
	0.3	13.25	25.49	37.74	49.98	62.23	74.47	86.72	98.97	111.21	123.4	
	0.2	63.4	125.8	188.2	250.6	313	375.4	437.8	500.2	562.6	625	
0.1	1,000.9	2,000.8	3,000.7	4,000.6	5,000.5	6,000.4	7,000.3	8,000.2	9,000.1	10,000		

with netting at one end. Mice were anaesthetised with Nembutal before being used as the source of a blood meal. We first counted the number of probes by individual uninfected and infected flies (previously starved for 24 and 48 h) which pierced the skin with the haustellum before they were gorged. Table 1 shows that infected flies probed significantly more frequently than uninfected control flies, and suggests that infected flies were more voracious, for all 40 infected flies took blood after 24 h whereas only 28 of 40 uninfected flies did so.

We then offered 10 infected flies (5 male, 5 female) a series of mice on which to probe. If an individual fly failed to engorge after probing and withdrawing its haustellum, it was offered another mouse. Of a total of 41 probes by 10 infected flies, 38 proved to be infective to mice; the highest number of infective probes by an individual fly was 6. There was no significant difference between male and female fly behaviour in the above experiment.

The labrum of two *T. (T.) brucei*-infected *G. m. morsitans* were successfully prepared for scanning electron microscopy (SEM) out of a total of eight flies with mature infections examined with the light microscope. All eight flies had groups of trypanosomes in the proximal part of the labrum and SEM examination of two infected labra demonstrated a specific association between parasites and LCI mechanoreceptors (Fig. 1). We know the subgenera *Nannomonas* and *Duttonella* colonise the labrum where LCI mechanoreceptors are located⁵ and are in close association with them^{9,10}.

We then applied principles of fluid mechanics^{11–15} to predict the behaviour of blood in the labrum of *Glossina* to evaluate the likely effects of colonies of trypanosomes on the rate of blood flow. The data, units and sources which allow calculations of the predicted effects of parasites on flow in this system are available from the authors. The flow is characterised by the Reynolds number¹², and a frequency parameter^{13–15} for the pulsating flow and non-newtonian viscosity effects are considered¹¹. From these calculations, the pressure drop ($-\Delta\bar{P}$) below atmospheric required to obtain the same flow rate, $\bar{Q}$, for the meal is obtained. Table 2 indicates the factors by which $-\Delta\bar{P}$ would need to be increased for a proportion of the length mL (for m , 0.1–1.0) infected with trypanosomes and the proportionate unoccluded diameter $n\bar{D}$ (for n , 0.1–1.0) if the same meal flow rate were to be obtained. Alternatively, if $-\Delta\bar{P}$ remained

constant, the flow rate would be inverse to the factors in Table 2. To illustrate the effect of occlusion due to *Nannomonas* and *Duttonella*^{9,10}, Table 2 shows that colonies of trypanosomes will be significantly invasive hydrodynamically, affecting blood flow rate through the labrum. Such changes would be detected by LCI mechanoreceptors⁵. Table 2 is also applicable to the trypanosome-infected hypopharynx, where a greater proportionate reduction in diameter would apply due to the smaller internal diameter of the hypopharynx; thus, the pressure required to expel saliva would be greater in infected than uninfected flies.

Table 3 The relationship between viscosity and feeding rate in *Glossina austeni* fed 24–48 h after emergence through bat or mouse skin membrane

Diet	Viscosity (cP)	Meal weight (mg)	Average feeding time (s)	Feeding rates (mg s ⁻¹)
10 ⁻³ M ATP saline	0.68	18	36	0.500
10 ⁻³ M ATP saline 3% dextran	1.36	18.6	48	0.387
10 ⁻³ M ATP saline 6% dextran	2.64	16.5	55.5	0.297
10 ⁻³ M ATP saline 12% dextran	6.78	18.1	82.25	0.220
Defibrinated sheep blood	3.10	16.5	63.3	0.26
Sheep plasma + 10 ⁻³ M ATP	1.15	16	37.1	0.431

Twenty flies were used for each diet and duration of feeding was measured electronically as described in ref. 6. Blood meal size was determined by weighing the fly before and immediately after feeding, before primary excretion took place. Viscosity was measured in a Cannon-Lebbelohde semi-microviscometer, size 200 at 38 °C

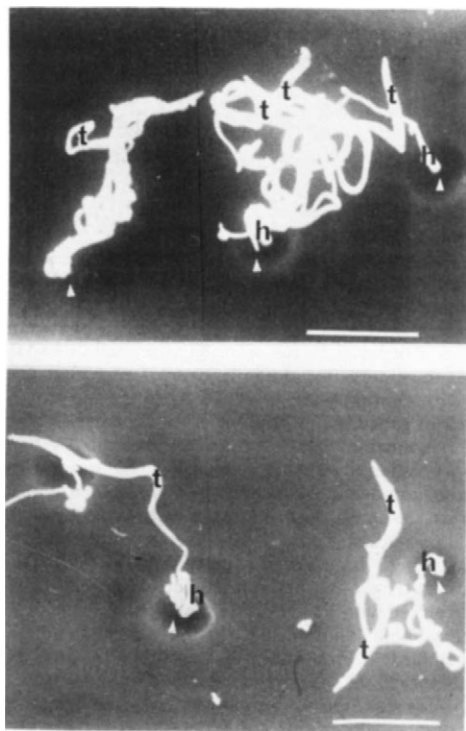


Fig. 1 Upper, scanning electron micrograph of *T. b. brucei* parasites (t, procyclic trypomastigotes) associated with two LCI mechanoreceptors (basal pits arrowed) in the proximal part of the labrum of *G. m. morsitans* 19 days after an infective feed. The preparation was fixed in 0.2 M glutaraldehyde in cacodylate buffer, critically point dried and examined in a Cambridge stereoscan. Scale bar, 10 μ m. Lower, two procyclic parasites (t) associated with one LCI mechanoreceptor in the labrum of *G. m. morsitans* (and a single parasite associated with a second LCI). Scale bar, 10 μ m.

However, if the viscosity of the blood meal was increased in membrane feeding experiments with *G. austeni*, there was a reduction in the rate of feeding of approximately 50% for a 10-fold increase in viscosity (Table 3). We have shown that there will be a need to increase either pump frequency or capacity by the factors given in Table 2 if the flow rate is to remain constant for any given occlusion in effective diameter by trypanosomes. However, we also know the pump performance is limited in its capacity to respond to an experimentally increased viscosity. Thus, to obtain the same blood meal size the infected fly would take longer to engorge or, if it did not take sufficient blood, would need to feed more frequently.

We know⁵⁻⁷ that the feeding and gorging responses of *Glossina* are determined by chemo- and mechano-sensilla located in the labellum, labrum and cibarium. The LR7 sensilla of the labellum initially detect a suitable food source in their role as ATP detectors; probing continues until there is (1) sufficient stimulus of the LCI mechanoreceptive sensilla of the labrum and of the LC2s of the anterior cibarial wall by an adequate blood flow rate, at which time (2) the four LC4 sensilla of the posterior cibarial wall are stimulated by the ATP present in the meal to begin the gorging phase of the feeding cycle. Thus, impairment of the LCI mechanoreceptor function will affect sensory input and modify the feeding behaviour of infected flies as described above. Such behaviour indicates an advantageous adaptation by parasites that may have profound epidemiological and epizootiological implications. In particular, the significance of salivarian trypanosome infection rates in *Glossina* will require re-examination if studies on the behaviour of different tsetse species in the field confirm our laboratory findings.

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Larval hatching from vitellogenin-deficient eggs developed in male hosts of the silkworm

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Egg production in insects is generally considered to require the availability of the yolk precursor, vitellogenin. In most insects, the synthesis of this protein in the female fat body and its uptake into the ovaries are under the control of the hormones, juvenile hormone and ecdysone¹, which is somewhat analogous to the oestrogenic control in oviporous vertebrates². Indeed, vitellogenin constitutes ~90% of total egg proteins in the cockroach, *Leucophaea maderae*³, and ~40% in the silkworm, *Bombyx mori* (unpublished data). However, in some insects⁴⁻⁹, rudimentary ovaries transplanted into male hosts are known to develop to mature eggs with chorion but lacking vitellogenin. *Drosophila melanogaster* is exceptional in that the ovaries themselves can produce vitellogenin, which accounts for its accumulation in ovaries transplanted into males⁸. Vitellogenin is believed to represent a reserve for use during embryogenesis^{10,11}. However, experiments on *D. melanogaster*¹² and *B. mori* (unpublished data) demonstrated that vitellin was not used rapidly during embryonic development and persisted at a considerable level in the newly hatched larvae. These facts suggest that vitellogenin may not be essential for ovarian development or for embryonic development. Therefore, the present experiments were carried out in order to establish whether the vitellogenin-deficient eggs that matured in male hosts, if activated by artificial parthenogenesis¹³, can compute embryonic and further post-embryonic development. We have found that they can.

Silkworms of a bivoltine race (Shunrei and Shogetsu) were programmed to produce diapause eggs by incubating the parental embryonic stage at 25 °C under continuous illumination. On the third day of the fifth instar, ovaries were transplanted into males, the testes of which had just been extirpated. The operated larvae metamorphosed into pupae in 9 days and into adults after a further 14 days, as did non-operated control animals. Mature eggs with chorion were taken from the newly emerged adults. Table 1 compares various characteristics of these eggs with those of eggs matured in females. The eggs from males were less numerous and slightly light in weight. The contents of amino acids, RNA and lipids were almost the same but the glycogen content was clearly higher in the eggs from males, and the protein content was about 20% lower than that of controls.

Polyacrylamide disc gel electrophoresis of the proteins of eggs from males demonstrated eight bands comparable with those

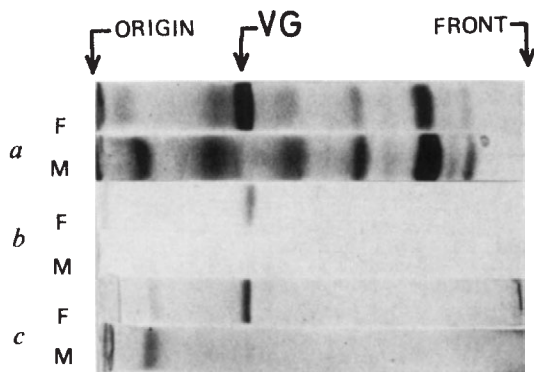


Fig. 1 Polyacrylamide gel electrophoresis of soluble proteins from eggs from females (F) and male hosts (M). Eggs (about 200 mg) obtained as described in Table 1 legend were homogenised in 5 vol of 0.4 M KCl in 50 mM phosphate buffer, pH 6.0. The homogenate was centrifuged at 15,000g for 15 min at 4 °C and 10–20 μ l of the supernatant containing 0.002% bromphenol blue and 20% glycerol was applied to a 5% polyacrylamide gel (pH 8.5) and run at a constant current of 2 mA per gel¹⁹. Gels were stained¹⁹ for protein with amido black 10B (a), for glycoprotein with Schiff's reagent (b), and for lipoprotein with Sudan black (c). VG, vitellogenin.

from normal eggs, but one normally major band corresponding to vitellogenin was lacking or at a trace level (Fig. 1). This was supported by staining for glycoprotein and lipid which are characteristic components of vitellogenin¹, and further by immunochemical assay (data not shown). Therefore, eggs from male hosts seem to differ from those produced in females chiefly in deficiency of vitellogenin.

To initiate embryogenesis in the eggs developed in male hosts, artificial parthenogenesis by a heat-shock treatment was carried out according to the method of Astaurov¹³, who applied it to eggs from ovaries in females. The parthenogenetic development was easily detected in eggs by a reddish-brown pigmentation which reflected the formation of the serosal cells and entry into diapause (Fig. 2). The treatment induced parthenogenetic development in 63.1 $\pm$ 16.2% of the eggs from male hosts, and in 59.7 $\pm$ 0.8% of normal eggs from females. Thus, there was no appreciable difference in capacity for parthenogenetic response.

Because these eggs were programmed for diapause, diapause was then prevented artificially in the usual way by HCl treatment (Fig. 2). Embryonic development progressed and larvae of hatching size appeared in about 21.0 $\pm$ 4.2% of the coloured

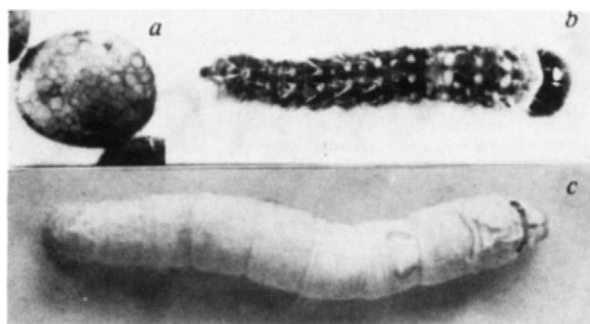


Fig. 2 Egg (a), newly hatched larva (b), and the fifth instar larva (c) from parthenogenetically activated eggs which had developed in male hosts. Ovaries dissected out from newly emerged males were immersed in water at 46 °C for 16 min and cooled abruptly in tap water for 10 min. After drying in air, the eggs were kept at 20 °C for 4 days to ensure parthenogenetic development¹³. The eggs with a reddish-brown colour were then soaked in HCl solution (specific gravity, 1.075 at 15 °C) at 47 °C for 3 min to prevent diapause²⁰. They were kept at 25 °C and 75% relative humidity thereafter. After 12 days, larvae hatched and were raised on mulberry leaves. Egg and newly hatched larva, $\times 20$; fifth instar larva, $\times 1.5$.

Table 1 Comparison of weight and composition of eggs developed in normal females and in male hosts

	Eggs developed in	
	Females	Male hosts
Wet weight (μ g per egg)	600 $\pm$ 30	556 $\pm$ 11
Dry weight (μ g per egg)	231 $\pm$ 1	188 $\pm$ 1
Amino acids (mg per g eggs)	2.3 $\pm$ 0.1	2.5 $\pm$ 0.2
RNA (mg per g eggs)	2.4 $\pm$ 0.8	3.0 $\pm$ 0.7
Lipids (mg per g eggs)	85.4 $\pm$ 2.4	87.8 $\pm$ 9.3
Glycogen (mg per g eggs)	26.5 $\pm$ 1.5	35.3 $\pm$ 2.5
Proteins (mg per g eggs)	105.2 $\pm$ 7.5	84.7 $\pm$ 10.7

Ovaries from fifth instar larvae were transplanted into males of the same stage. At the emergence of adults, ovaries were dissected out and mature eggs with chorion were collected by squeezing from ovarioles through a layer of gauze. After brief air drying, the eggs were weighed, then dried three times at 80 °C for 3 h to constant dry weights and weighed again. Fresh eggs were homogenised in cold water with an agate mortar and the homogenates were passed through a cotton pad to remove chorion residues. The filtered sample was used for determination of amino acids, RNA, lipids, glycogen and proteins. Amino acids were extracted three times with cold 5% HClO₄. After neutralisation with KOH, the solution was applied to a column of Dowex-50 (H⁺ form) and eluted with 1 M NH₄OH. The pooled eluate was used for determination of total amino acids by the ninhydrin reaction, with leucine as standard¹⁴. From the acid-insoluble residues RNA was extracted with hot 5% HClO₄ and determined by the orcinol reaction, with yeast RNA as standard¹⁵. Protein was extracted from the residues with 0.5 M NaOH and determined by the method of Lowry *et al.*¹⁶, with bovine serum albumin as standard. Lipids were extracted from fresh homogenates with methanol/chloroform (3:1) and the evaporated residues were then extracted with chloroform, washed with 0.5 M NaCl, dried and weighed¹⁷. For glycogen, the homogenate was digested with 30% KOH at 100 °C for 30 min, and glycogen was precipitated with 4 vol of ethanol overnight. In some experiments a cold HClO₄ soluble fraction was used directly for glycogen determination. Glycogen was determined with the phenol-sulphuric acid or concentrated H₂SO₄ method with glucose as standard¹⁷. Values are means $\pm$ s.e.m. from three to five determinations on different pools of eggs (about 350 eggs, 200 mg).

eggs 8 days after the HCl treatment. Hatching occurred in about 5.7 $\pm$ 2.7% of the parthenogenetic eggs, which is comparable with that in normal eggs from females¹³. The larvae appeared to be rather pale in colour but moved actively. When raised on fresh mulberry leaves, the larvae moulted to the second instar 4 days later, continued to grow successfully and reached maturity to spin cocoons on day 25 at 25 °C.

We believe that the completion of embryogenesis and subsequent larval growth from the eggs that developed in male hosts is the first such case reported in insects as well as in other classes of animals. The results demonstrate that vitellogenin or vitellin is not essential for egg maturation and embryonic development. In addition, such a biological system should open a new field in reproductive and embryonic studies.

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Noradrenaline and cyclic AMP—-independent growth stimulation in newt limb blastemata

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Adult newts regenerate functional limbs after amputation^{1,2}. This process normally depends on the trophic influence of nerves on the regenerating limbs, particularly in the early stages before differentiation of the regeneration blastema³, when it stimulates growth by maintaining high rates of macromolecular synthesis⁴. The sequence of biochemical events involved is unknown, but it has been suggested that intracellular cyclic AMP may be a second messenger within the blastema⁵⁻¹⁰. Many studies have indicated that the neural agent(s) involved might be protein^{4,11-16}. The recent finding that blastemata contain high levels of catecholamines¹⁷, however, has implicated noradrenaline (NA) as the neurotrophic agent, and suggested that it works via stimulation of β -adrenergic receptors on the blastemal cells, thereby raising the intracellular concentrations of cyclic AMP¹⁰. To test this hypothesis we studied the ability of NA alone and in combination with α - and β -adrenergic antagonists to increase cyclic AMP levels and to mimic the effects of nerves by maintaining high rates of protein synthesis^{13,14} and high mitotic indices (MI)^{15,16} in denervated blastemata in organ culture. We find that although NA raises cyclic AMP levels through a β -adrenergic effect, it does not maintain high rates of protein synthesis or high MI in cultured blastemata. It is unlikely therefore, that this hypothesis applies.

The rate of protein synthesis and the mitotic index in blastemata decrease after denervation^{3,11}. Similar changes occur after blastemata are denervated and explanted into organ culture¹³⁻¹⁶. The addition of extracts of newt brains to the culture medium mimics the trophic effects of nerves by preventing these post-denervation decreases¹³⁻¹⁶. We cultured cone stage blastemata¹⁸ from newts (*Notophthalmus viridescens*) in the presence of brain extracts, NA, and NA together with either an α - or β -adrenergic antagonists and determined the effects of these treatments on the cyclic AMP levels¹⁰ rates of protein synthesis^{13,14} and MI^{15,16} in the blastemata. Histological examination revealed a normal, viable regenerate¹⁶. In confirmation of our previous findings, blastemata cultured in the presence of chick embryo or adult newt brain extracts had rates of protein synthesis and mitotic indices that were significantly greater than those in control blastemata cultured in their absence. In contrast to the effects of brain extracts on the blastemata, NA (at concentrations ranging from 10^{-6} to 10^{-4} M) did not elevate the MI and caused a small but significant decrease in the rates of protein synthesis below control values (Fig. 1). This indicates that, under the present experimental conditions, contrary to the hypothesis proposed by Taban *et al.*¹⁰, it is unlikely that NA is an important neurotrophic agent since it does not mimic at least two important neurotrophic effects: maintenance of high rates of protein synthesis and high MI in the blastemata.

Unlike NA alone, NA in combination with the β -adrenergic antagonist propranolol had no effect on the rate of protein

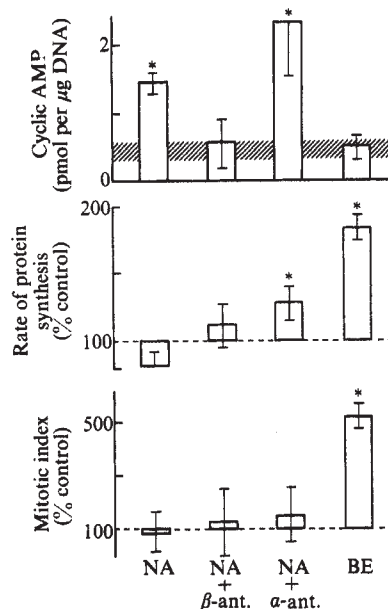


Fig. 1 Effects of NA, adrenergic antagonists and brain extracts on the cyclic AMP concentration, mitotic index, and protein synthesis of blastemata cultured *in vitro*. Cone stage blastemata were explanted and cultured as described previously¹⁴⁻¹⁶. Control cultures contained no addition to the medium; there were four treatment groups; NA = medium containing 10^{-4} M NA; NA + α -ANT = medium containing 10^{-4} M NA + either 10^{-4} M phenoxybenzamine or 10^{-4} M phentolamine; NA + β -ANT = medium containing 10^{-4} M NA + 10^{-4} M propranolol; BE = medium containing an aqueous extract of adult newt brain (0.3 mg ml^{-1}) or embryonic chick brain (1.0 mg ml^{-1}) (refs 14-16). Blastemata were cultured in the presence of the various agents and the levels of cyclic AMP (at 10 min), rates of protein synthesis (at 12 h) and MI (at 24 h) were determined. At these time periods, the maximal effects of the treatments on various parameters occurred. Each value is the mean $\pm$ s.e.m. of six determinations. Cyclic AMP was determined using a radioimmunoassay²² as modified by Harper and Brooker²³. The rate of protein synthesis in the blastemata¹⁴ and mitotic indices^{15,16} were determined as described elsewhere. Differences in cyclic AMP concentrations, mean MI and rate of protein synthesis between experimental blastemata and controls from contralateral limbs were analysed for significance by the *t*-test for non-independent samples²⁴. * $P \leq 0.05$. The cross-hatched area on the cyclic AMP graph represents control values ± 1 s.e.m. The control values were as follows: MI = $0.64 \pm 0.06\%$ (s.e.m.). Rate of protein synthesis = 2.33 ± 0.05 (s.e.m.) pico atoms of carbon per g protein per 12 h. Cyclic AMP = 0.478 ± 0.06 (s.e.m.) pmol per μg DNA.

synthesis in the blastemata, or on their MI, both of which were the same as in control cultures (Fig. 1). Conversely, the addition to the cultures of NA plus either of the α -adrenergic antagonists, phenoxybenzamine or phentolamine, significantly increased the rate of protein synthesis in treated blastemata, while MI was unaffected. The increase in protein synthesis due to this β -adrenergic effect was far smaller however, than that produced by brain extracts (Fig. 1). This small increase in protein synthesis observed when the α -adrenergic effects of NA are inhibited, leaving only the β -adrenergic effects, is of particular interest. It is unlikely that the selective stimulation of β -adrenergic receptors is responsible for the neurotrophic effects on blastemata *in vivo* however, since there is no evidence that naturally occurring α -adrenergic antagonists exist. Furthermore, when the β -adrenergic effects of NA are blocked, protein synthesis in blastemata is not inhibited. It appears, therefore, that the inhibitory effects of NA on the rate of protein synthesis may be due to the combined simultaneous activation of α - and β -adrenergic receptors.

Although NA stimulated increases in cyclic AMP in cultured blastemata (and thus confirmed the report of Taban *et al.*¹⁰), it failed to stimulate increases in the rates of protein synthesis or

mitosis, two neurotrophically regulated processes of the nerve-dependent growth phase of limb regeneration^{3,11}. Previous studies have shown that the effects of brain extracts on protein synthesis and mitosis in cultured blastemata are similar to the effects of nerves on these processes *in vivo*^{14,16}. Thus, the failure of NA to mimic the neurotrophic effects of brain extracts *in vitro* on these two processes would argue against this catecholamine as an important trophic effector during the early stages of regeneration. The possibility remains, however, that NA may play a significant role during the later nerve-independent stages of redifferentiation. Indeed, pharmacological studies on regeneration *in vivo* support such an hypothesis⁷; treatment with anti-adrenergic agents does not prevent blastema formation (a nerve-dependent process) but retards or represses subsequent morphogenesis (a nerve-independent process).

Additionally, crude brain extracts were ineffective in stimulating increased cyclic AMP levels in cultured blastemata. These results demonstrate that the elevation of intracellular cyclic AMP in blastemal cells is not invariably associated with increases in the rates of protein synthesis and mitosis in the blastemata. Consequently, the previously described stimulation of DNA and protein synthesis and mitosis by exogenous dibutyl cyclic AMP^{5,16,19} may be mediated through a different mechanism.

We have previously found that stimulation of α -adrenergic receptors increases cyclic GMP levels in newt tissues, and that β -adrenergic stimulation inhibits such increases²⁰. It has been suggested that the ratio of cyclic AMP to cyclic GMP may play an important role in the response of tissue muscle to neurotrophic influences^{10,21}. If true, this might explain the observed failure of NA to elevate protein synthesis and MI. This interpretation is not supported by observations of the effects of denervation on the levels of cyclic nucleotides in blastemata *in vivo* (M.P.R. Liversage and McLaughlin, unpublished observations); it appears likely that any alterations in cyclic AMP and cyclic GMP levels after denervation are not induced directly by the neurotrophic stimulus, but rather reflect a more general metabolic alteration in the blastemata that is part of the overall metabolic response associated with the increased growth stimulated by the neurotrophic influence.

Thus our findings are inconsistent with the hypothesis¹⁰ that nerves transmit trophic information to blastemal cells via β -adrenergic receptor mediated stimulation of adenylate cyclase with subsequent production of cyclic AMP. Studies are currently in progress to determine the nature of the neurotrophic influence and to elucidate the molecular mechanisms involved in the response of blastemata to neurotrophic stimuli.

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Cigarette smoke-induced DNA damage and lung cancer risks

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Epidemiological studies have firmly established that cigarette smoking causes almost all cases of anaplastic and squamous cell bronchial carcinomas¹. It is equally well known, however, that many smokers do not develop lung cancer and, although the amount of tobacco smoked is undoubtedly the dominant risk factor, it seems possible that other factors may also be involved². There is increasing evidence to support Boveri's³ old hypothesis that somatic mutation is an important event in the development of cancer^{4,5} and we have recently shown that cigarette smoke condensate (CSC) produces many dose-related lesions in the cellular DNA of cultured human lymphocytes as measured by sister chromatid exchange (SCE) induction⁶. Cytogenetic studies^{7,8} had previously shown an increase in chromosomal aberrations in blood lymphocytes of heavy smokers relative to non-smokers, but little⁹ or no^{8,10} increase in SCEs. We have now measured basal and CSC-induced SCE rates in lymphocytes from different individuals and report that smokers have higher rates than non-smokers and that smokers with untreated lung cancer have consistently higher rates than their matched heavy smoking controls. These results are in keeping with the somatic mutational hypothesis and the epidemiological evidence, but also raise questions related to difficulties in smoking dosimetry and to possible variation among different individuals' responses to a common insult.

The responses of cultured lymphocytes from four groups of people to four doses of smoke condensate were examined by the visual scoring of induced SCEs. We used the same methods as in our original report⁶ and, as before, samples were coded and scored 'blind'. Smoke condensate produced from a standard brand of British cigarette was dissolved in dimethylsulphoxide at serial dilutions and stored in small aliquots at -20°C . This condensate, together with single stocks of culture medium and fetal calf serum, were used throughout the series. Four cultures, each of 10 ml and containing 0.8 ml of whole blood with an average of 10^6 lymphocytes, were set up from each individual. The four groups studied were: *A*, 10 healthy smokers, none of whom to our knowledge had been unduly exposed to any mutagen other than cigarette smoke. *B*, 10 healthy non-smokers who were examined in pairs matched for age and sex with members of group *A*. *C*, 12 patients with histologically proven but untreated non-disseminated anaplastic or squamous cell bronchial carcinomas, in whom diagnostic biopsies had been obtained by fiberoptic bronchoscopy under local anaesthetic. Ten of these patients were continuing to smoke at the time of testing (*C*₁), but two had stopped smoking 2 and 5 yr previously (*C*₂). *D*, 10 control patients with other disorders, but none with any form of cancer, who were on average 10 yr older and had smoked similar numbers of cigarettes per day, but for 10 yr longer, than our lung cancer patients. All individuals in groups *C* and *D* were inhaling smokers and smoked their cigarettes to comparable butt lengths. Only one individual had been exposed to a recognised occupational risk factor (asbestos). All were hospital inpatients, none of whom was acutely ill, and individuals from both groups had received similar exposure to X rays and drugs.

From our results, shown in Tables 1 and 2, it may be seen that cigarette smoke condensate (CSC) present in small quantities

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Table 1 Frequency* of sister chromatid exchanges in cultured lymphocytes from healthy smokers and non-smokers exposed to cigarette condensate (CSC)

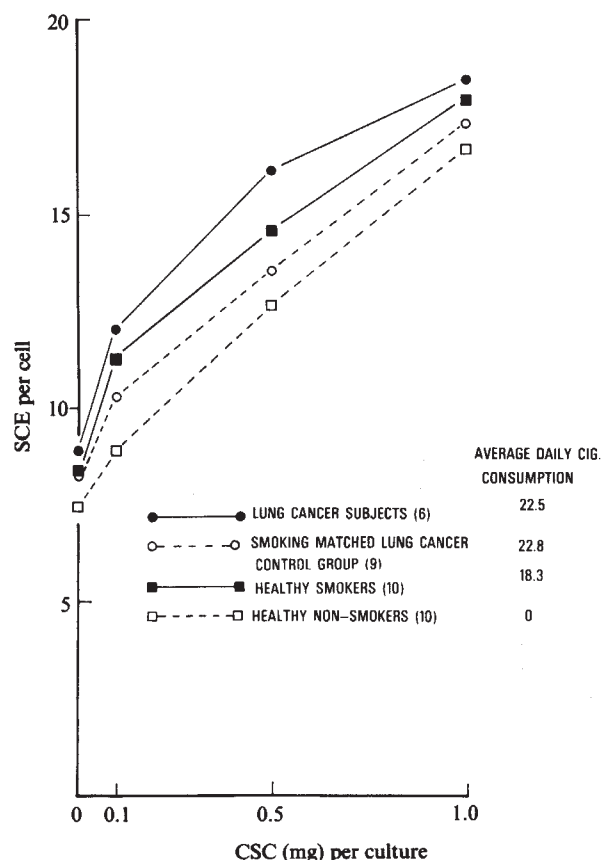
	Age	Sex	Cigarettes per day	Dose of CSC†			
				0	0.1	0.5	1.0
B Non-smokers	47	M		7.5	9.4	11.6	13.6
	45	F		7.2	8.4	11.1	16.8
	29	F		7.1	8.0	12.7	15.8
	24	M		7.8	9.5	11.6	16.0
	21	M		7.5	7.6	12.7	15.8
	75	M	(ex-smoker of 17 yr)	7.0	9.9	12.2	18.3
	73	F		7.0	9.1	12.7	19.4
	59	M		7.2	8.2	15.1	18.0
	48	M		8.5	9.5	12.8	15.2
	54	F		7.5	9.1	14.0	17.9
A Smokers	47.6			7.43 (0.14)	8.87 (0.24)	12.65 (0.38)	16.68 (0.55)‡
	47	M	15	6.6	9.7	13.4	15.4
	54	F	20	7.3	11.7	14.1	17.7
	32	F	20	8.5	11.9	15.3	19.0
	24	M	15	8.2	10.5	14.0	16.5
	20	M	15	6.8	10.0	12.8	15.8
	70	M	10	8.0	13.8	17.0	22.3
	75	F	12	10.5	11.9	16.2	—
	64	M	10	6.5	7.5	11.1	16.9
	53	M	35	9.4	12.0	15.1	17.5
	54	F	30	12.0	14.6	17.5	20.1
	49.3		18.3	8.38 (0.57)	11.36 (0.65)	14.62 (0.64)	17.91 (0.74)§
				9.30 (0.89)	12.55 (0.65)	15.5 (0.72)	18.53 (0.60)
				7.76 (0.62)	10.57 (0.79)	14.08 (0.89)	17.38 (1.26)¶

* Mean of 20 cells per culture.

† CSC, mg per 10 ml culture containing 10^6 lymphocytes (diluent, dimethyl sulphoxide 20 μ l). Note: 0.1 mg CSC = 1/400 of smoke from one high tar cigarette; 0.5 mg CSC = 1/80; 1.0 mg CSC = 1/40.

‡ Mean (s.e.) of individual means.

§ Mean (s.e.) overall.

|| Mean (s.e.) ≥ 20 cigarettes per day.¶ Mean (s.e.) < 20 cigarettes per day.**Fig. 1** Within group pooled mean SCEs (that is, the mean of individuals' means), at different dose levels of CSC, for the various groups.

produced dose-related increases in SCEs in lymphocytes from all individuals. Basal and induced SCE rates did, however, differ significantly among individuals.

Healthy smokers (A) had, relative to healthy non-smokers (B), higher mean basal and also higher induced SCE rates (following exposure to 0.1 mg of CSC, $P < 0.05$) and the heaviest smokers had the highest SCE rates. A comparative study on cultured whole blood cells of two smokers and two non-smokers showed that these differences between smokers and non-smokers are maintained even in the absence of autologous plasma; we conclude, therefore, that the greater number of SCEs produced by condensate in smokers' cells in comparison with non-smokers must reflect some cellular alteration dependent on previous exposure to smoke *in vivo*.

For patients with lung cancer who were continuing to smoke (C_1), SCE rates were significantly higher at all levels of testing than for the smoking matched cancer controls (D). During final analysis, we found that there were more plain cigarette (middle or high tar) smokers at the time of testing in our lung cancer group than in the matched controls and this might have introduced a bias. However, exclusion of these plain cigarette smokers from both groups does not influence the result and smoking lung cancer subjects continue to have higher SCE rates at all levels of testing (0.1 or 0.5 mg of CSC, $P < 0.01$). The two ex-smokers with lung cancer (C_2) had responses indistinguishable from those of healthy non-smokers, suggesting that high SCE rates are not secondary to the development of cancer. Furthermore, the overlapping of responses between the healthy smokers (A), the smoking matched non-cancer patients (D) and the smoking lung cancer patients (C_1), supports this conclusion.

Figure 1 presents mean basal and induced SCE frequencies for filter cigarette smokers in groups A, C and D (that is, smokers exposed to comparable levels of smoke *in vivo*) and for

Table 2 Frequency of sister chromatid exchanges in cultured lymphocytes from patients with lung cancer and matched smoking controls exposed to CSC

	Age	Sex	Cigarettes per day	Cigarette type & smoking duration		Dose of CSC			
				Plain	Filter	0	0.1	0.5	1.0
C ₁ Lung cancer subjects	60	M	40	47	—	12.4	14.1	16.5	21.6
	51	M	25	36	—	9.6	12.2	16.8	20.4
	66	M	20	58	—	12.4	15.8	21.0	24.6
	51	M	25	36	—	12.3	14.0	20.3	20.1
	63	M	30	36	20	8.5	12.4	15.5	19.8
	55	F	20	23	15	7.3	13.2	14.3	16.3
	63	F	20	25	15	9.9	10.8	15.9	20.4
	65	M	25	39	12	8.3	13.8	19.0	20.3
	53	F	20	18	20	9.8	11.4	16.5	17.8
	45	M	20	—	25	9.8	11.3	15.6	16.5
	56.9		24.5	42.3 yr		10.03 (0.57)	12.9 (0.49)	17.14 (0.70)	19.73 (0.78)*
						11.7 (0.69)	14.03 (0.74)	18.65 (1.17)	21.67 (1.02)†
						8.93 (0.44)	12.10 (0.40)	16.13 (0.64)	18.43 (0.75)‡
C ₂ Lung cancer subjects (ex-smokers of 2 and 5 yr)	57	M	30	20	20	7.3	9.5	10.4	12.7
	56	M	20	20	11	7.5	9.1	12.0	14.4
D Smoking matched cancer control group	56	M	30	42	—	10.6	13.9	18.0	19.2
	65	M	20	40	12	8.7	10.8	13.8	—
	85	M	20	55	20	8.6	10.1	12.9	—
	73	M	30	43	20	7.2	10.8	13.9	17.0
	61	F	20	28	15	9.4	11.2	13.2	16.7
	64	F	20	34	15	8.6	10.6	15.9	21.9
	73	M	25	44	15	7.7	8.3	13.1	18.4
	58	F	20	33	10	8.6	11.4	13.1	15.0
	71	M	30	56	2	6.9	8.6	12.2	15.7
	53	M	20	17	20	8.8	11.1	13.8	16.8
	65.9		23.5	52.1 yr		8.51 (0.33)	10.68 (0.49)	13.99 (0.54)	17.59 (0.77)§
						8.28 (0.27)	10.30 (0.38)	13.54 (0.34)	17.35 (0.85)

* Mean (s.e.) overall.

† Plain cigarette smokers at testing.

‡ Filter cigarette smokers at testing.

§ Mean (s.e.) overall.

|| Filter cigarette smokers at testing.

non-smokers (*B*) and shows specifically that there is a consistent ordering of our four groups at all levels of testing. Superficially, the almost parallel dose-response curves in Fig. 1 might imply that the increased SCE rates following *in vitro* induction may simply emphasise *in vivo* differences, with similar increments being added for all four groups for each *in vitro* unit dose. This cannot be ruled out, but at lower CSC doses the relative response is not uniform between groups and more data would be needed to distinguish between these two possibilities. More important, however, is the fact that *in vitro* induction highlights the differences between the four groups. Highest responses were found for smokers with lung cancer (*C*₁). Among smokers, the lowest responses were found for the older heavy-smoking non-cancer patient group (*D*), and this is in keeping with our selection of these individuals as a 'relatively low risk' group. An unselected group of healthy and younger smokers (*A*) who might reasonably be expected to contain individuals of varying risk for lung cancer show a mean response intermediate between the lung cancer subjects (*C*₁) and their controls (*D*). This association of risk with extent of DNA damage supports the possibility that somatic mutation may be important in initiating malignant transformation in cells.

Although we have shown that cigarette smoking and the number and type of cigarette smoked significantly influence SCE rates, it is also clear that smokers seemingly exposed to similar amounts of cigarette smoke *in vivo* can have significantly different SCE rates (groups *C*₁ and *D*). These differences might simply result from discrepancies in actual amounts of tobacco smoked—certainly, all smoking histories describe only alleged amounts—or from varying patterns of inhalation or of airflow in the bronchial tree, both of which would clearly influence the

dose of carcinogen delivered to bronchial mucosa and peripheral lymphocytes. Alternatively, if real smoke dosage *in vivo* was equivalent in both groups—and there is no way of determining this precisely—these differences might reflect the influence of other factors on the extent of measured DNA damage. In the context of Tokuhata and Lilienfield's¹¹ finding of significant familial aggregation of lung cancer not extending to spouses, it seems possible that some of these factors might be genetic and this would not be surprising for complex events resulting in DNA exchange, where presumably many enzymatic processes are involved, particularly in a species like man, where high rates of protein polymorphism are known to exist¹².

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Peptide-containing neurones connect the two ganglionated plexuses of the enteric nervous system

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The enteric nervous system (ENS) of the mammalian gut consists principally of two ganglionated plexuses, the myenteric and submucous, which are embedded in the gut wall¹. Little is known about the anatomical and functional connections between the two plexuses and further clarification of their relationship is of central importance for the understanding of the ENS. In the present study we have approached this problem in a new way, using methods we have previously developed for maintaining the myenteric and submucous plexuses separately *in vitro* for several weeks^{2,3}. In an immunohistochemical study of these preparations and also of sections from normal and extrinsically denervated gut wall, we have found evidence for reciprocal projections between the myenteric and submucous plexuses, by nerve fibres containing two putative neurotransmitters, vasoactive intestinal polypeptide (VIP)⁴ and substance P (ref. 5) (see Fig. 1). Our observations were supported by radioimmunoassay of tissue extracts. These results suggest that one of the roles of these peptides in the gut is to coordinate the function of the two enteric plexuses.

The normal distribution of VIP and substance P immunoreactivity in sections through the wall of the guinea pig caecum underneath and including the taenia coli is illustrated in Fig. 2. The VIP antiserum revealed a dense network of mostly varicose nerve fibres surrounding the ganglion cells of the myenteric plexus. Fibres were also frequent in the longitudinal and circular muscle, running parallel to the long axis of the muscle bundles, as well as in the ganglia of the submucous plexus. In the submucosa outside these ganglia and in the mucosa, VIP immunofluorescent fibres were present, but infrequent. Occasionally immunofluorescent nerve cell bodies were seen in the ganglia of the submucous plexus. The distribution and density of substance P immunoreactivity was similar to that of VIP, except that the density of substance P immunofluorescent fibres in the ganglia of the myenteric plexus was somewhat higher than that of VIP fibres. Substance P immunofluorescent fibres were not seen in the mucosa and no nerve cell bodies were detected with certainty.

It is recognised that neuronal perikarya of peptide-containing neurones are difficult to visualise, even if the axons to which they give rise contain demonstrable peptide stores^{6,7}. To identify unambiguously the intrinsic source of the peptide-containing fibres we used our ability to immunostain tissue culture preparations of the myenteric and submucous plexus. The plexuses were removed from the gut wall and maintained separately *in vitro* for 1–2 weeks as described previously^{2,3}. This enabled us to study peptide immunoreactivity in varicose axons which grow out from the neurones of each plexus during the culture period; these plexuses are free of axons extrinsic to the gut and also of those intrinsic axons, which *in situ* might run between them. Immunostaining of the cultures revealed that no VIP

immunofluorescence could be detected in the myenteric plexus, whereas explants of submucous plexus contained numerous intensely fluorescent varicose fibres, which could often be traced for long distances in the outgrowth zone around the explants, as well as being observed among the neuronal cell bodies inside the explant (Fig. 3a). Conversely, substance P immunofluorescence was absent from the explants of the submucous plexus but was found in abundance in varicose fibres of the myenteric plexus cultures, both in the outgrowth zones and inside the explants (Fig. 3b). It is unlikely that our inability to observe VIP immunoreactivity in the myenteric plexus cultures is due to the failure of VIP-containing neurones to grow *in vitro*, or to technical difficulties in demonstrating VIP stores in nerve fibres in culture, because of the large numbers of VIP fluorescent fibres found in the cultures of the submucous plexus. The same argument applies to the absence of substance P immunoreactivity from the submucous plexus cultures. We therefore suggest

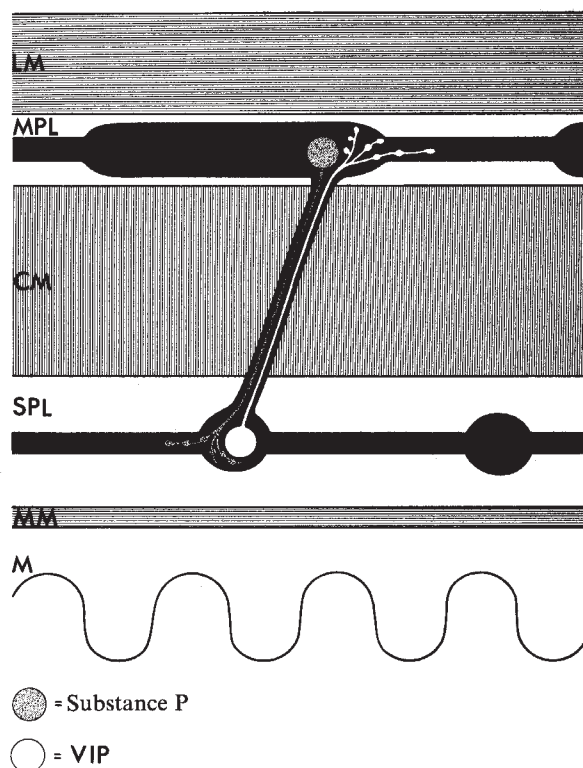


Fig. 1 Schematic drawing of a section through the wall of the caecum including the taenia, showing VIP- and substance P-containing neurones connecting the myenteric and submucous plexuses. LM, longitudinal muscle—this muscle layer constitutes the taenia coli. MPL, myenteric plexus—one whole ganglion and part of another are illustrated together with interconnecting strands. CM, circular muscle—the longitudinal and circular muscle layers are together referred to as the external muscle layer. SPL, submucous plexus—two ganglia and interconnecting strands are shown. MM, mucosal muscle. M, mucosa.

that the guinea pig caecum contains intrinsic systems of both VIP- and substance P-containing neurones, the intrinsic VIP fibres originating exclusively from cell bodies located in the submucous plexus and the intrinsic substance P fibres emanating only from cell bodies in the myenteric plexus.

In the intact gut, however, we found VIP and substance P fibres densely surrounding cells in the myenteric and submucous plexuses respectively. There are two alternative explanations for these observations: (1) these fibres are extrinsic, originating from cell bodies outside the gut; or (2) the VIP fibres in the

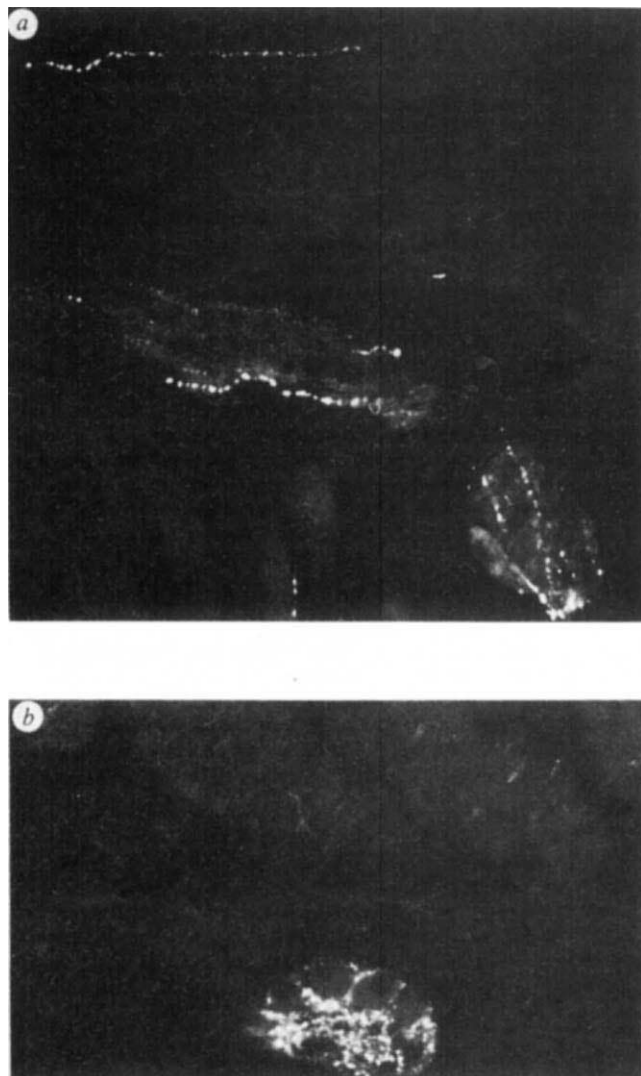


Fig. 2 Immunostaining of nerve fibres in sections from the caecum. *a*, VIP antiserum; *b*, substance P antiserum. In (*a*) immunoreactive beaded fibres can be seen among non-immunoreactive cell bodies in two ganglia of the myenteric plexus. A long beaded fibre is also visible in the longitudinal muscle above the ganglia and a short segment of a stained fibre can be seen associated with transversely cut bundles of the circular muscle below the ganglia ($\times 175$). In (*b*) immunoreactive fibres can be seen surrounding non-reactive neuronal cell bodies in a ganglion of the submucous plexus. Above the plexus faint immunoreactivity can be seen in transversely cut fibres in the muscle bundles of the circular muscle layer ($\times 250$). Both VIP and substance P antibodies were raised in rabbits using purified porcine VIP or synthetic substance P coupled to bovine serum albumin by the carbodiimide method. The VIP antibody was directed to the C-terminal of the molecule and was used at a dilution of 1/400. The substance P antibody was directed to the whole molecule and was used at a dilution of 1/2,000. Neither antibody showed any significant cross-reactivity with other known neuropeptides. Staining was carried out by the indirect immunofluorescence method¹⁴. Tissue cultures or 10- μ m thick cryostat sections of benzoquinone-fixed¹⁵ specimens from the caecum were incubated with the peptide antibodies overnight at 4°C. A second layer of fluorescent isothiocyanate conjugated anti-rabbit globulin was applied for 1 h at room temperature. In control experiments sections or cultures were incubated with nonimmune serum or VIP and substance P antisera which had been incubated with purified VIP or substance P respectively; no immunostaining was observed in these experiments.

myenteric plexus originate from the submucous plexus and the substance P fibres in the submucous plexus originate in the myenteric plexus. To distinguish between these possibilities we denervated the caecum of extrinsic nerve fibres by crushing the mesenteric nerves with fine forceps 3 weeks before killing, or, in some cases, by freezing them with a freeze probe. The effectiveness of the denervation was ascertained by noting the disappearance of the extrinsic adrenergic fibres from the gut wall (see ref. 8). No change in the amount or distribution of VIP or

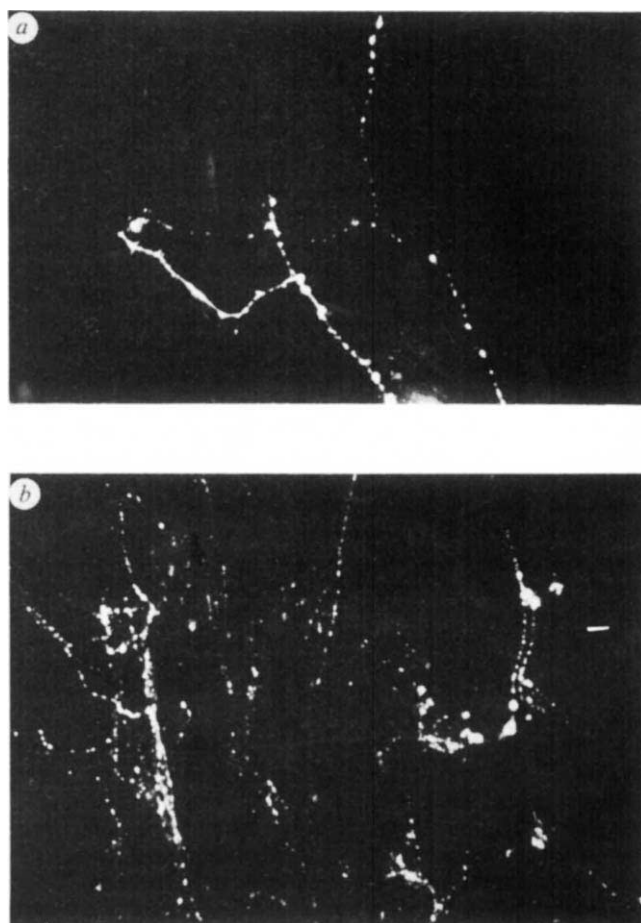


Fig. 3 Immunostaining of nerve fibres in tissue culture preparations of the enteric plexuses. *a*, VIP antiserum applied to a culture of the submucous plexus; intensely stained, apparently single, beaded nerve fibres, lying on top of non-immunoreactive, flattened, non-neuronal cells in the outgrowth zone of a culture which had been maintained for 7 days *in vitro* ($\times 700$). *b*, Substance P antiserum applied to a culture of the submucous plexus; several immunostained nerve fibres lie either apparently singly or in bundles in the outgrowth zone of a culture which has been maintained *in vitro* for 11 days. Under the nerve fibres lie flattened non-neuronal cells which show no immunoreactivity ($\times 500$).

substance P immunofluorescent fibres was seen in the ganglia of the enteric plexuses in four experiments in spite of complete disappearance of adrenergic fibres. Furthermore, no significant change in VIP content, as measured by radioimmunoassay⁹, was observed when denervated segments of gut wall underneath and including the taenia were compared to normally innervated segments from the same animal or from other control animals; the values for normal segments were 14.8 ± 4.2 pmol per g wet weight of tissue ($n = 6$) and for denervated segments 12.7 ± 4.3 pmol per g wet weight of tissue ($n = 4$). It is conceivable that nerve fibres may reach the caecum by running inside the gut

wall, originating either from extrinsic nerves which enter the gut above or below the caecum or from the enteric plexuses of the small intestine or proximal colon, although the existence of such fibres has never been demonstrated. These fibres would not be affected by our denervation procedures. We therefore made a circumferential cut through the external muscle of the guinea pig caecum in the region just below the site of entry of the small intestine and proximal colon. The incision extended to the submucosa without penetrating it. In this way all nerve fibres which might reach the myenteric plexus of the caecum via the muscle layers or via the myenteric plexus were severed. The animal was killed 18 days later and sections from the caecum were immunostained, revealing VIP and substance P immunostaining which was identical both in distribution and density to that of normal material. Taken together these experiments demonstrate that the caecum receives no significant extrinsic innervation by VIP- or substance P-containing fibres.

We thus conclude that the submucous plexus of the caecum contains VIP neurones which provide most or all of the VIP innervation found in all layers of the intestinal wall in this area of the gut, including the dense network of fibres in ganglia of the myenteric plexus. In contrast, the substance P immunoreactive fibres of the gut wall, which are present in highest density in the ganglia of the myenteric and submucous plexuses, originate mostly or exclusively from the neurones located in the myenteric plexus. We have shown that in the caecum no VIP fibres originate from the myenteric plexus, and no substance P fibres emanate from the submucous plexus. This provides evidence for a VIP-containing pathway running from the submucous plexus to innervate neurones in the myenteric plexus as well as for a substance P-containing pathway providing innervation by the myenteric plexus of the submucous plexus as shown schematically in Fig. 1. A suggestion that there might be nerve connections between the two plexuses has been made previously on the basis of light microscopical findings¹⁰, and has since received some support from physiological experiments¹¹. Note, for comparison, that enkephalin immunofluorescent fibres do not span the two ganglionated plexuses but originate in the myenteric plexus and are confined to its ganglia and the external muscle layer^{12,13}. A detailed account of the distribution of VIP, substance P and enkephalin neurones in the guinea pig small and large intestine is in preparation¹³.

Although variations of the pattern described here may exist in other parts of the gut (for instance, some VIP-containing fibres originate from the myenteric plexus of the ascending colon and small intestine¹³) and in other species, these observations warrant pharmacological and physiological investigations into the possibility that one of the roles of VIP- and substance P-containing neurones in the gut is to provide functional connections between the two enteric plexuses.

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Substance P increases membrane conductance in parotid acinar cells

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Substance P, a naturally occurring polypeptide of mammalian origin¹, has been implicated both as a neurotransmitter and a neurohormone². However, little is known of the ionic mechanisms underlying the postsynaptic response to substance P. In myenteric neurones Katayama and North³ reported substance P-evoked depolarisations (of varying latency) associated, surprisingly, with a decrease in membrane conductance. A direct measurement of reversal potential in normal ionic conditions was not achieved but it was suggested that substance P acts by reducing membrane potassium conductance. In contrast, work on salivary glands suggests that substance P evokes an increase in potassium conductance^{4,5}; however, electrophysiological work has not been carried out to verify this. We report here that substance P evokes a marked increase in rat parotid acinar cell membrane conductance associated with a potential change (latency 1.7 s) that reverses at about –65 mV. The reversal potential for substance P is shown to be identical to that obtained in the same cells for acetylcholine (ACh) and adrenaline. The identical membrane action of ACh, adrenaline and substance P, mediated by three separate receptor sites, suggests activation of a common effector mechanism.

Isolated segments of rat parotid gland were mounted in a tissue bath through which oxygenated physiological saline solutions were circulated at 37 °C. Single microelectrodes were used to record both membrane potential and input resistance of surface acinar cells⁶. Substance P (Peninsula Lab.) was applied both by iontophoresis^{7,8} and by addition to superfusion media. The iontophoretic application of ACh and adrenaline has been described previously⁹.

The mean resting membrane potential was -73.3 ± 0.8 mV ($\pm$ s.e.m., $n = 62$) and the mean input resistance of the unstimulated cell was 4.8 ± 0.3 M Ω ($\pm$ s.e.m., $n = 38$). Responses to iontophoresis of substance P were recorded in 42 cells from six rats. In 21 of these cells, responses to iontophoresis of ACh were also recorded, and in 12 responses to iontophoresis of three agonists, substance P, ACh and adrenaline, were recorded. Figure 1 shows the similar nature of the responses to the three stimuli in a single cell. Each agonist evokes a marked decrease in input resistance associated with an initial depolarisation. This depolarisation is followed by a delayed hyperpolarisation during which the input resistance returns to the resting level. At less negative resting membrane potentials the initial potential change becomes a hyperpolarisation, but in any one cell the responses to all three stimuli are the same. Such biphasic potential changes have been reported previously in response to iontophoresis of the two autonomic agonists⁹. Only the substance P response was resistant to autonomic blockade by a combination of atropine (10^{-6} M), phentolamine (10^{-5} M) and propranolol (5×10^{-6} M). Responses were recorded in 11 cells and in the presence of all three blockers.

Figure 2 demonstrates the marked reduction in input resistance induced by substance P even in the presence of autonomic blockade. Although there was no detectable change in either the magnitude or duration of the substance P response on introduction of the blockers, the latency of the response was affected. In the control solution the latency of the response was 608 ± 122 ms (mean $\pm$ s.e.m. $n = 6$). On introduction of the blockers this latency became $1,737 \pm 165$ ms (mean $\pm$ s.e.m., $n = 4$),

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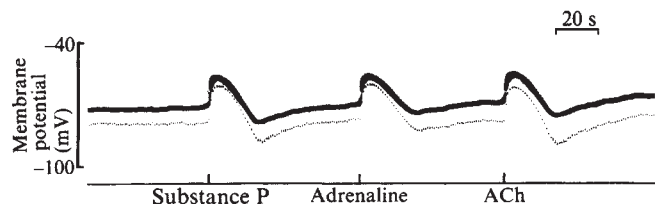


Fig. 1 Recording of membrane potential and input resistance from acinar cell of rat parotid. Responses are seen to iontophoretic application of substance P, adrenaline and ACh. The substance P micropipette contained 3.5 mM of peptide in 20 mM acetic acid (pH 5–7). The ejection current was 200 nA applied for 500 ms. Responses were recorded to ejection charges as low as 10 nC. Iontophoresis of a control solution (20 mM sodium acetate acidified to pH 5.7) never evoked any response. Adrenaline iontophoresis was achieved at 500 nA for 200 ms; ACh at 100 nA for 1 s. Hyperpolarising current pulses (2 nA, 100 ms) were repetitively injected through the recording microelectrode to obtain input resistance.

suggesting that in the absence of blockade an indirect effect of substance P is manifested at least as a shortened latency. The latency of the direct effects is nevertheless one of the shortest so far recorded for iontophoresis of substance P, variable latencies of between 1 and 30 s having been reported previously^{3,7}.

Responses could also be evoked by inclusion of substance P in the superfusion media in concentrations of 10^{-7} to 10^{-9} M. Changing the superfusion medium to one containing the drug induced a marked decrease in input resistance, this time associated with a slow monophasic hyperpolarisation. Such responses were obtained in nine cells from eight rats. In five experiments the superfusion medium also contained all three autonomic blockers.

Application of substance P (15 nmol) directly to the tissue bath as a single-shot injection (0.1 ml) evoked responses resembling those induced by iontophoresis. The similar, even repetitive, application of either of two other active polypeptides caerulein or bombesin (0.1 ml containing 1.5 pmol and

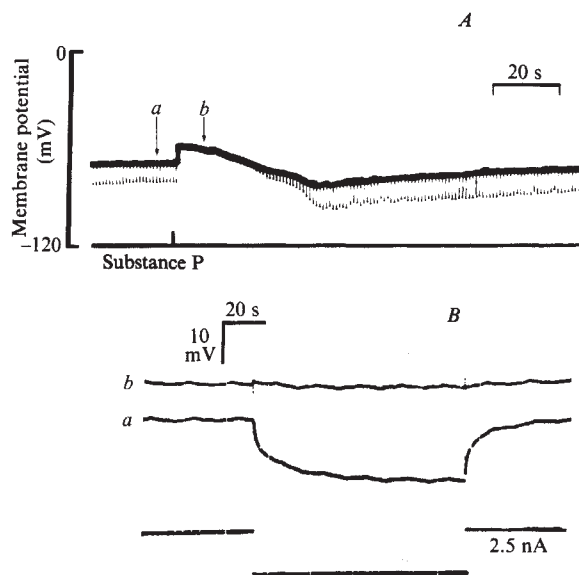


Fig. 2 Substance P response in the presence of full autonomic blockade; that is atropine (10^{-6} M), phentolamine (10^{-5} M) and propranolol (5×10^{-6} M). *a*, Response to iontophoresis of substance P (200 nA for 500 ms); *b*, oscilloscope picture showing amplitudes of the electrotonic potential changes due to injection of current pulses (2.5 nA hyperpolarising, 100 ms) at times *a* and *b* indicated in trace *A*. This demonstrates the marked reduction in input resistance at point *b* as a consequence of the iontophoretic application of substance P. This decrease in input resistance is not due to rectification as the current-voltage relationship of these cells is linear as previously shown in mouse parotid⁹.

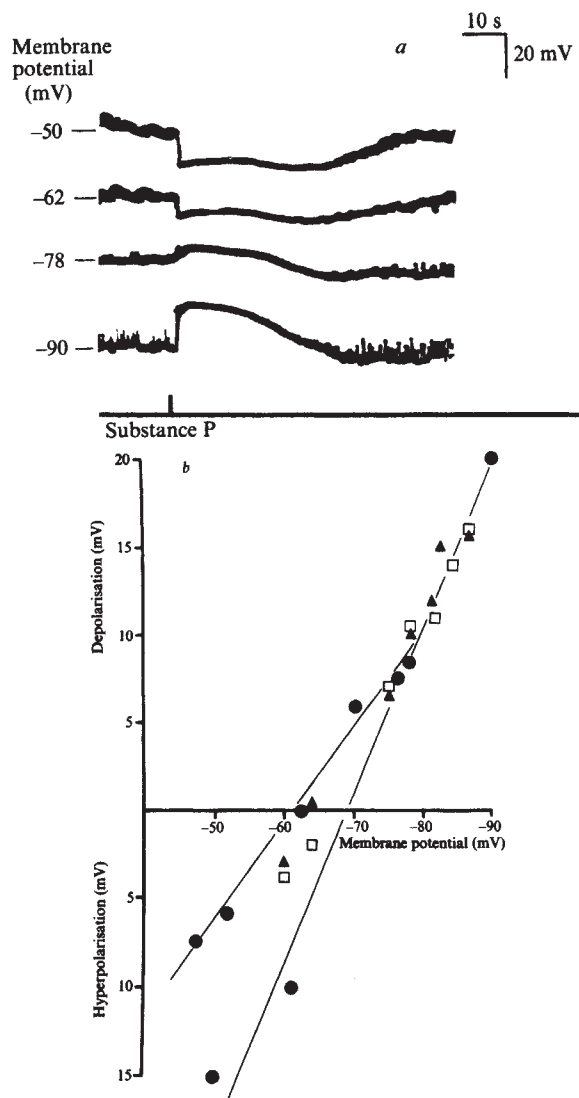


Fig. 3 *a*, Family of traces showing the nature of the substance P response at different levels of membrane potential in the same cell. The membrane potential is varied by applying direct current through the recording electrode. The superfusion medium contained all three autonomic blockers. The response changed from a depolarisation to a hyperpolarisation (reversal) as the membrane potential became less negative. In *b* the amplitudes of substance P responses are plotted as a function of membrane potential (●). Two plots are shown for substance P responses from different preparations and these are compared with plots of ACh (▲) and adrenaline (□) responses in a third preparation. The reversal potentials of the responses, the intersection with the abscissa, for all three stimuli are between -60 and -70 mV. (Substance P, 100 nA for 1 s; ACh, 100 nA for 1 s; adrenaline 500 nA for 1 s.)

3.0 pmol, respectively) failed to evoke a response in any of 12 cells from five preparations. These concentrations are known to be effective in evoking responses from pancreatic acinar cells¹⁰. This demonstrates that the peptide receptor is not accessible to all active polypeptides and is in agreement with the previous finding¹¹ that these gastrointestinal hormones cannot evoke salivary secretion.

The reversal potential of the substance P response was determined by two methods. Trautwein and Dudel¹² describe how the 'transmitter equilibrium potential' can be determined by analysis of the changes in both membrane potential and input resistance induced by an agonist. This analysis was carried out for 58 responses to substance P, 35 responses to ACh and 17 responses to adrenaline. The mean reversal potentials were: substance P, -64.4 ± 0.9 mV ($\pm$ s.e.m.); ACh, -64.5 ± 1.3 mV ($\pm$ s.e.m.); adrenaline -65.7 ± 2.2 mV ($\pm$ s.e.m.). The values of

the reversal potential for substance P and adrenaline had not been previously determined, but the ACh reversal potential is in very close agreement with the value of -63 mV previously reported¹³.

Figure 3 demonstrates the second method used. The amplitude of the responses from a single cell is recorded at different levels of membrane potential. The amplitude of the response is then plotted as a function of membrane potential and the intersection with the abscissa represents the reversal potential. Figure 3b shows two plots of responses to substance P and compares them with responses to ACh and adrenaline. Responses to all three stimuli undergo reversal between -60 and -68 mV. Thus both methods of estimating reversal potential give comparable values and illustrate that the reversal potentials for all three stimuli are the same, that is about -65 mV.

Our observations reveal that in a salivary gland substance P evokes changes in membrane potential and input resistance which are resistant to autonomic blockade, probably due to direct activation of a peptide receptor. A degree of specificity of the peptide receptor is indicated by the inability of two other active polypeptides, caerulein and bombesin, to evoke any response. The ionic mechanisms underlying the acetylcholine response has been extensively investigated¹³. The demonstration that substance P and adrenaline share the same reversal potential strongly suggests that responses to the three stimuli, although mediated by separate receptors, are the consequence of activation of the same effector mechanism primarily an increased potassium and sodium conductance.

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Dual action of ototoxic antibiotics on sensory hair cells

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All aminoglycoside antibiotics in clinical use are ototoxic in that they can cause permanent malfunctioning of the cochlea and vestibule¹⁻³. It is generally assumed that these ototoxic antibiotics primarily affect the sensory hair cells in the inner ear and that degeneration of secretory and neural elements comes later²⁻⁵. Most experimental work on aminoglycoside antibiotic ototoxicity has concentrated on the irreversible effects on inner ear functioning and hair cell morphology^{2,3}. Little is known about their mode of action on the hair cells. Interference with hair cell protein synthesis, in analogy with the antimicrobial mechanism, has been ruled out as the primary cause of the high selective toxicity of the aminoglycosides^{3,5}. Recent evidence indicates that these antibiotics may interfere with membrane phospholipid metabolism⁷. Morphological studies have demonstrated that early on in the ototoxic process the membrane of the

sensory hairs is already damaged⁴. The aminoglycoside antibiotics are reported to have a direct and reversible^{8,9} effect on hair cell functioning in organs of the acoustico-lateralis system of lower vertebrates⁸⁻¹¹. Hence, the primary site of action is probably the hair cell membrane. We have now therefore examined reversible effects of ototoxic antibiotics on single fibre afferent nerve activity and extracellular receptor potentials in the lateral-line organ of *Xenopus laevis*. Our results show that the antibiotics, in concentrations similar to those measured in the cochlear fluids³, have a dual action on the hair cells. They not only increase the spontaneous afferent nerve activity, probably by an effect on the hair cell membrane, but also severely impair the mechano-electric transduction process, resulting in a large phase lag of the receptor potentials. The latter effect, which is antagonised by Ca^{2+} , may be due to interference of the antibiotics with the mechanical properties of the sensory hairs.

The experiments were carried out on isolated preparations of the lateral-line organ of the clawed frog, *X. laevis*. The organ was stimulated by small-amplitude sinusoidal water movements. Activity of single afferent nerve fibres was computed in period histograms, from which gain and phase were determined as previously described¹². Amplitude and phase of extracellular receptor potentials were measured as described elsewhere¹³. The effects of the antibiotics were constant within 5 min of application and for concentrations up to $25 \mu\text{g ml}^{-1}$ were reversible.

Figure 1a shows that dihydrostreptomycin in concentrations between 2.5 and $17.5 \mu\text{g ml}^{-1}$ enhanced spontaneous afferent nerve activity. An increase in spontaneous afferent activity has recently been predicted by Gallais *et al.*¹¹ from behavioural observations on frogs injected in one ear with streptomycin. It is tempting to conclude that the transient symptom of tinnitus,

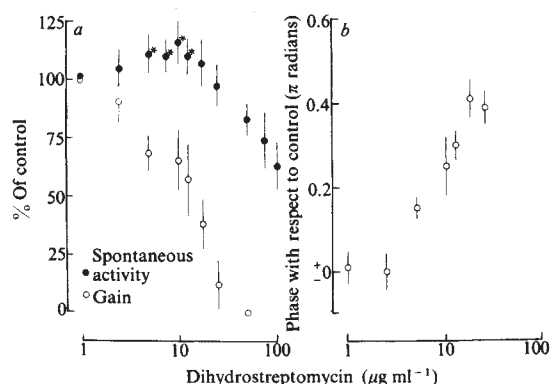


Fig. 1 Effect of different concentrations of dihydrostreptomycin (K & K Laboratories) on spontaneous afferent activity (a), and on gain (a) and phase (b) of the afferent nerve response to stimulation of the lateral-line organ of *Xenopus laevis*. A piece of skin containing several lateral-line organs was placed in an experimental chamber. The inner side of the skin was in contact with Ringer's solution, the outside was covered with tap water, which was replaced every 15 min. Temperature was 18°C . Each 15-min period with tap water was followed by a 15-min period with fresh tap water to which the antibiotic had been added. Controls were taken before and after each period in which the antibiotic was applied. Stable control values could be obtained from the preparations for a period of more than 2 h. At 7 and 12 min, respectively, after each replacement of the tap water, the lateral-line organ was stimulated for 30-70 s with local, sinusoidal water movements (20 Hz) induced by a small glass sphere. Period histograms of single fibre afferent nerve activity were computed and analysed by means of Fourier analysis. The gain was expressed as per cent modulation of mean afferent activity per μm sphere displacement. The phase of the response was measured with respect to sphere displacement and expressed in radians. Spontaneous activity was measured before each stimulation over 1,000 intervals as the mean number of impulses per s. Further details of recording, stimulation and data analysis can be found elsewhere¹². The values for spontaneous activity and gain obtained in the presence of dihydrostreptomycin were expressed as a percentage of control. The phase of the response in the presence of dihydrostreptomycin was expressed as phase shift with respect to control. Spontaneous activity was measured in 31 preparations; each point represents the mean $\pm$ s.d. of 10 measurements. Gain and phase were measured in 12 preparations; each point represents the mean $\pm$ s.d. of six measurements. Asterisk denotes spontaneous activity significantly higher than control (Student's *t*-test; $P < 0.05$).

which is known to occur soon after administration of ototoxic antibiotics¹, is due to an increase in spontaneous afferent activity of the auditory nerve fibres. Additional experiments showed that blocking of the cholinergic efferent synapse¹⁴ by curare or atropine (5×10^{-5} M) did not prevent the increase in spontaneous activity induced by $10 \mu\text{g ml}^{-1}$ dihydrostreptomycin. Thus, it is unlikely that the increase in spontaneous activity was brought about by an effect of the antibiotics on the efferent synapse¹⁵. Apparently, the antibiotics act directly on the hair cell membrane and cause an increase in spontaneous transmitter release¹⁶ from the hair cells.

With higher concentrations of dihydrostreptomycin ($>17.5 \mu\text{g ml}^{-1}$), spontaneous activity was depressed (Fig. 1a). Depression of afferent activity by aminoglycoside antibiotics has been demonstrated previously in the mammalian cochlea^{2,3} and in the semicircular canal organ of the frog^{9,11}. This effect is consistent with other known effects of high concentrations of aminoglycoside antibiotics, that is, the inhibition of transmitter release^{17,18} and postsynaptic blockade^{19,16} in the neuromuscular junction, and interference with action potential generation in frog myelinated nerve²⁰.

The afferent nerve response to stimulation at 20 Hz was markedly depressed by dihydrostreptomycin (Fig. 1a). The gain decreased gradually with increasing concentration of the antibiotic and $50 \mu\text{g ml}^{-1}$ abolished response to stimulation. In addition, dihydrostreptomycin caused a progressive phase lag in the afferent nerve response up to a value of 0.4π radians at $25 \mu\text{g ml}^{-1}$ (Fig. 1b). Figure 1 clearly illustrates the dual action of dihydrostreptomycin: low concentrations increased the spontaneous activity, but at the same time impaired the mechanical sensitivity.

Other aminoglycoside antibiotics produced a similar dual effect. Streptomycin was about as effective as dihydrostreptomycin (maximum increase in spontaneous activity at 7.5 and $10 \mu\text{g ml}^{-1}$, respectively), whereas gentamycin was somewhat less effective (maximum increase at $17.5 \mu\text{g ml}^{-1}$). Detailed results will be published elsewhere.

Figure 2 shows that dihydrostreptomycin induced a decrease in amplitude and a phase lag of the receptor potentials almost identical to that measured in the afferent nerve response. A similar phase lag of the receptor potentials was observed at 1 and

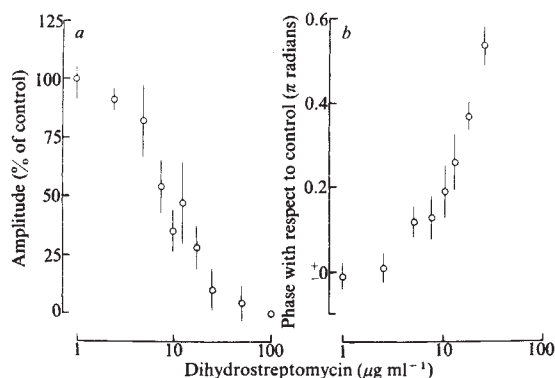


Fig. 2 Effect of different concentrations of dihydrostreptomycin on amplitude (a) and phase (b) of extracellular receptor potentials recorded from the sensory hair cells in one neuromast of the lateral-line organ of *Xenopus laevis*. Temperature was 22°C . Preparation and application of the antibiotic were as described in Fig. 1 legend. At 7, 10 and 13 min, respectively, after each tap water replacement, the neuromast was stimulated for 60 s with short sequences of sinusoidal water movements (20 Hz; 8 periods per sequence). Extracellular receptor potentials were recorded by means of Pt-Ir microelectrodes. The response to 30 stimulus sequences was digitised, averaged and plotted. Amplitude and phase with respect to sphere displacement of the extracellular receptor potentials were computed by means of Fourier analysis of the averaged response. Details of recording, stimulation and data analysis can be found elsewhere¹³. Values for amplitude and phase of the response obtained in the presence of dihydrostreptomycin were expressed, respectively, as a percentage of control and as phase shift with respect to control (radians). Results were obtained from nine preparations; each point represents the mean $\pm$ s.d. of six measurements.

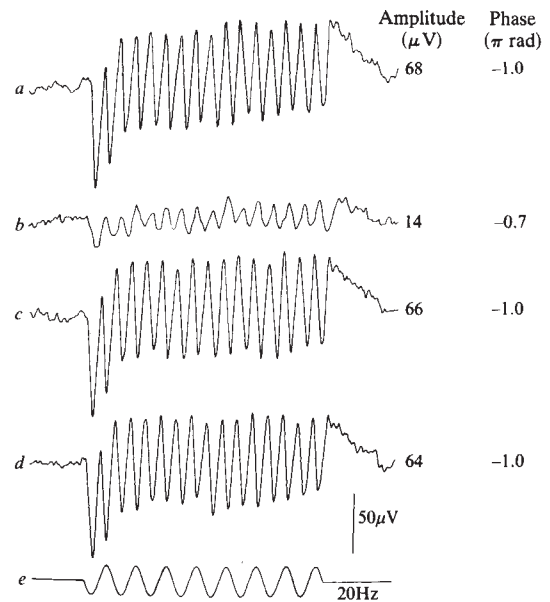


Fig. 3 Prevention of the effect of dihydrostreptomycin on extracellular receptor potentials from the lateral-line organ of *Xenopus laevis* by 5 mM CaCl_2 . Preparation and application of the antibiotic were as described in Fig. 1 legend. At 13 min after each tap water replacement, the neuromast was stimulated for 60 s with short sequences of sinusoidal water movements (20 Hz; eight periods per sequence) induced by a small glass sphere. Each trace is the averaged response to 30 stimulus sequences. The lowest trace (e) denotes the displacement of the glass sphere. a, Control; b, during application of $17.5 \mu\text{g ml}^{-1}$ dihydrostreptomycin; c, during application of $17.5 \mu\text{g ml}^{-1}$ dihydrostreptomycin and 5 mM CaCl_2 ; d, control. The control recording between b and c has been omitted. Amplitude and phase with respect to sphere displacement were computed by means of Fourier analysis of the averaged response. To exclude any transient effects, the first two stimulus periods of the averaged response were discarded. Values for amplitude (μV) and phase (radians) are shown on the right side of each trace. Details of recording, stimulation and data analysis can be found elsewhere¹³. The normal Ca^{2+} concentration in the tap water was about 1 mM. Addition of 5 mM CaCl_2 alone had no noticeable effect on the receptor potentials.

5 Hz. The similarity between the effects of dihydrostreptomycin on the afferent nerve response and on receptor potentials demonstrates that the antibiotics primarily affect the hair cells and not the afferent nerve fibres. Further, the antibiotics did not affect the sinusoidal shape of the afferent nerve response¹² or the characteristic dead time in the spontaneous activity.

Aminoglycoside antibiotics are known to interfere with Ca^{2+} -dependent membrane phenomena^{7,17,21}. In the present study, the effects of dihydrostreptomycin on receptor potentials were fully prevented by 5 mM CaCl_2 (Fig. 3). There is evidence that Ca^{2+} ions are essential for hair cell transduction^{22,23}, although their precise role remains unclear^{23,24}. It has been suggested that Ca^{2+} modulates the permeability of the hair cell membrane to K^+ ions²⁴, which are thought to carry the receptor current^{24,25}. However, the time delay corresponding to the phase lag, particularly at low stimulus frequencies, is too large to be accounted for by an effect on the conductance mechanism underlying the receptor current.

A possible explanation for such a large phase lag is that the antibiotics alter the mechanical properties of the sensory hair bundle. Aminoglycoside antibiotics can cause sensory hair fusion and it has been postulated that they reduce the fixed negative charge on the sensory hair membrane, thereby diminishing the electrostatic repulsion between the hairs^{4,26}. The effects reported here were easily reversible, which implies that we have studied an early stage in the ototoxic process. It is conceivable that before sensory hair fusion the antibiotics increase the coupling between the sensory hairs, resulting in a reduced mobility of the hair bundle. An alternative hypothesis is that the antibiotics affect the stiffness of the individual hairs, which depends on their fibrillar core, rather than on their membrane²⁶. As the filaments in the core are composed of

actin²⁷, it can be speculated that the antibiotics alter their mechanical properties by interfering with Ca²⁺. Further work on the action of aminoglycoside antibiotics may lead to a better understanding of the role of Ca²⁺ in the transduction process.

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Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens

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Several drugs, including clofibrate (ethyl-*a-p*-chlorophenoxyisobutyrate)¹, are now available for the treatment of hyperlipidaemias^{2,3} and others are in the process of preclinical or clinical evaluation^{4,5}. Clofibrate, the most widely used hypolipidaemic drug in Europe and the US², as well as other potent hypolipidaemic agents, cause massive hepatomegaly when administered to rats^{6–8}, mice^{7,8} or hamsters (J.K.R., unpublished). This hepatomegaly is characteristically associated with a marked increase of peroxisomes (Fig. 1) in the liver cells of all three species^{6–8}. These ubiquitous cytoplasmic organelles^{9,10} contain catalase, several hydrogen peroxide-generating oxidases, carnitine acetyltransferase¹¹ as well as enzymes involved in the β -oxidation of long-chain fatty acids¹². The activities of these enzymes in liver are elevated in association with peroxisome proliferation^{8,13}. As hepatomegaly and peroxisome proliferation persist for as long as these drugs are administered to the animals, we initiated chronic toxicity studies with selected hepatic peroxisome proliferators in CS^b mice and F344 rats. Liver tumours were observed in both rats and mice fed nafenopin (2-methyl-2[*p*-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]propionic acid)^{14–16} or Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid)¹⁷, two structurally unrelated

compounds (Fig. 2, compounds 2 and 3) which are several times more potent than clofibrate in inducing peroxisome proliferation and hypolipidaemia⁸. Recent evidence indicates that clofibrate (Fig. 2, compound 1) is also carcinogenic when fed to rats at a concentration of 0.5% in the diet^{18,19}. We report here that BR-931 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio(*N*- β -hydroxyethyl)-acetamide)]²⁰ and tibrac acid (2-chloro-5-(3,5-dimethylpiperidinylsulphonyl)benzoic acid)⁸, two potent hypolipidaemic hepatic peroxisome proliferators (Fig. 2, compounds 4 and 5) induce hepatocellular carcinomas in CS^b mice and/or F344 rats. Thus, the development of liver tumours, in animals fed five structurally diverse hypolipidaemic compounds (Fig. 2) supports our hypothesis that potent hepatic peroxisome proliferators as a class are carcinogenic.

The cumulative data in Table 1 indicate that BR-931 given at 0.2% dietary level caused a significant increase in the incidence of liver tumours in female CS^b mice and male F344 rats. The tumours were multiple and measured 0.5–3.5 cm in diameter.

Table 1 Cumulative data on liver tumour incidence in female CS^b mice and/or male F344 rats fed hypolipidaemic hepatic peroxisome proliferator BR-931 or tibrac acid

Group	Sex	Treatment	No. of animals		No. of animals with liver tumours (%)
			Initial	Effective*	
CS ^b mice	F	BR-931 (0.2% for 19 months)	15	12	11† (92)
CS ^b mice	F	—	15	15	0 (0)
F344 rats	M	BR-931 (0.2% for 16 months)	20	20	20‡ (100)
F344 rats	M	BR-931 (0.05% for 19 months)	10	10	7† (70)
F344 rats	M	Tibrac acid (0.2% for 7.5 months, 0.1% for 4 months and 0.05% for 5 months: total 16.5 months)	35	31	30‡ (97)
F344 rats	M	—	30	30	— (0)

CS^b mice (4–6 weeks of age) derived from a colony maintained in our laboratory (JKR) and F344 rats (weighing 60–90 g) obtained from ARS, Sprague-Dawley, were used. The hypolipidaemic drugs BR-931 (LPB Istituto Farmaceutico) and tibrac acid (Pfizer) were added to powdered chow at the concentrations (w/w) shown and fed *ad libitum*. Autopsies were carried out on dead or moribund animals. All surviving animals at the end of the treatment periods shown were killed under light ether anaesthesia.

* The effective number of animals was the number of survivors at the time the first tumour was observed.

† *P* (one-sided) < 0.001 when compared with the controls by Fisher's exact test.

‡ *P* < 0.001 when compared with the controls by χ^2 test corrected for continuity.

Histologically, these tumours were hepatocellular carcinomas, some of which metastasised to the lungs. BR-931 at 0.05% level induced tumours in 7 of 10 rats; there were few tumours per liver when compared with the liver tumour load in rats fed the higher dose. Tibrac acid treatment of rats as outlined in Table 1 also resulted in a significant increase in the incidence of hepatocellular carcinomas. No liver tumours were encountered in the control groups of mice or rats. The ultrastructural appearance of BR-931- and tibrac acid-induced hepatocellular carcinomas in rats and mice was characterised by the presence of many peroxisomes in tumour cells, as noted previously in liver tumours induced by nafenopin¹⁴ or Wy-14,643 (ref. 17).

There was a significant increase in carnitine acetyltransferase activity in the tumours and normal portions of tumour-bearing livers in the BR-931 fed rats, but as expected¹⁸, the activity of peroxisomal marker enzyme catalase was not elevated (data not shown). Similar enzyme changes were previously observed in liver tumours induced by the peroxisome proliferator Wy-14,643 (ref. 17).

These experiments form part of an investigation to test the hypothesis that hypolipidaemic drugs capable of inducing hepatic peroxisome proliferation are carcinogenic. The experiments reported here with BR-931 and tibric acid and previous studies with nafenopin^{14,16}, Wy-14,643 (ref. 17) and clofibrate^{18,19} strongly favour this hypothesis, which can be tested further experimentally because of the availability of several other potent hypolipidaemic agents possessing hepatomegaly and peroxisome proliferative properties. This group comprises structurally diverse chemicals which share unique biological properties, including the ability to induce marked hepatomegaly, hepatic peroxisome proliferation and hypolipidaemia.

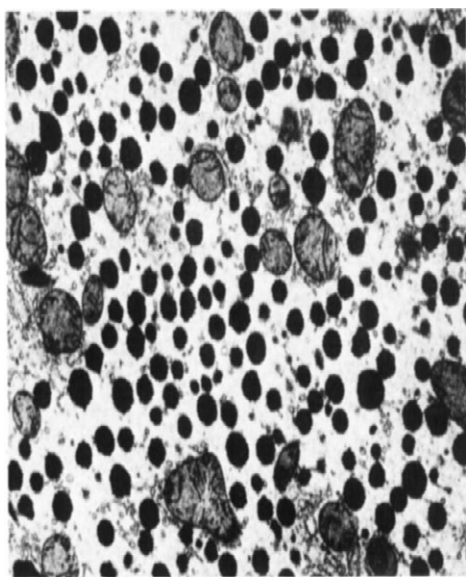


Fig. 1 Treatment with hypolipidaemic drugs causes proliferation of peroxisomes in liver parenchymal cells of rats, mice and hamsters. Many peroxisomes (electron dense bodies) are present in this electron micrograph, which represents a portion of a liver cell of a male rat fed a hypolipidaemic drug at a dietary concentration of 0.1% (w/w) for 4 weeks. The hepatomegaly and peroxisome proliferation persist for as long as the drug is administered. The hypothesis that chemicals capable of inducing hepatic peroxisome proliferation are carcinogenic is supported by the observation that five structurally diverse peroxisome proliferators are carcinogenic in mice and/or rats.

For these reasons, the hepatic peroxisome proliferators may be considered as a unique class of chemical carcinogens. Furthermore, unlike most known chemical carcinogens which inhibit DNA synthesis²¹, the carcinogenic hepatic peroxisome proliferators identified thus far seem to stimulate DNA synthesis and cell division in intact liver without causing any antecedent necrosis^{8,17,20}. Finally, none of these compounds caused detectable mutagenic activity in the *Salmonella*/microsome assay, or produced DNA damage in the lymphocyte ³H-thymidine incorporation assay²². The mechanism by which these drugs induce liver tumours and the role, if any, of peroxisomal enzymes in, for example, excessive production and/or breakdown of H₂O₂ (ref. 17) or hypolipidaemia, and in the initiation of carcinogenesis such as that invoked for lysosomal DNase in inducing chromosomal aberrations²³, remain to be examined.

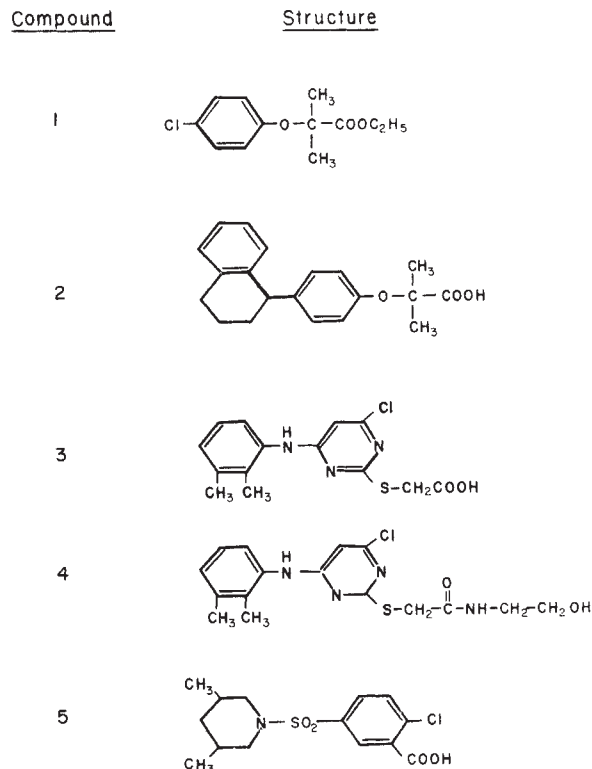


Fig. 2 Chemical structures of selected hypolipidaemic hepatic peroxisome proliferators tested for carcinogenicity. Compound 1: ethyl- α -p-chlorophenoxyisobutyrate (clofibrate, Atromid-S); Compound 2: 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]propionic acid (nafenopin); Compound 3: [4-chloro-6-(2,3-xylydino)-2-pyrimidinylthio]acetic acid (Wy-14,643); Compound 4: [4-chloro-6-(2,3-xylydino)-2-pyrimidinylthio(N-B-hydroxyethyl)-acetamide] (BR-931); Compound 5: 2-chloro-5-(3,5-dimethylpiperidin-2-ylsulfonyl)benzoic acid (tibric acid). All these compounds induced a significantly high incidence of hepatocellular carcinomas in mice and/or rats.

Whatever the mechanism(s) involved, the morphological finding of hepatic peroxisome proliferation seems a good indication for selecting a potential hypolipidaemic chemical for long-term carcinogenicity testing.

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Viroid synthesis: the question of inhibition by actinomycin D

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Viroids, pathogenic, low molecular weight (10^5) single-stranded circular RNA molecules, represent a unique class of minimal infectious agents. Physical characterisation has culminated in the primary sequence of the 359 nucleotides comprising the potato spindle tuber viroid (PSTV)¹. However, other than the dramatic expression of disease symptoms in plants², little is understood of the synthesis and biological activity of the viroid RNA. Molecular hybridisation techniques resulted in the detection of both viroid complementary RNA³ and DNA⁴ sequences. The putative template role of the DNA sequences was reinforced by the reported inhibition of viroid synthesis by actinomycin D⁵⁻⁷. However, viroid-specified proteins do not seem to function in replication or pathogenesis, for *in vitro* translation has not been accomplished^{8,9}. Furthermore, no AUG initiation triplets have been found in the primary sequence of PSTV or in the hypothetical complementary sequence. Viroid synthesis, therefore, may be characterised as a markedly host-dependent process and consequently potentially very sensitive to inhibition of DNA-directed functions by agents such as actinomycin D. The accumulation of viroid progeny in nuclei^{5,10} and nuclear-rich subcellular fractions¹¹ has provided clues to the mechanism of viroid synthesis. What was reported as a viroid complementary DNA sequence from DNA-rich extracts of *Citrus exocortis* viroid (CEV)-infected tissue⁶ has subsequently been positively identified as complementary RNA³ that may be associated with DNA-rich preparations. With further purification and treatment, no significant levels of viroid-complementary DNA can be detected in diseased *Gynura aurantiaca* or tomato. Therefore, we feel an RNA intermediate must assume a predominant role in any scheme for CEV replication and/or pathogenesis. We now report that synthesis of CEV and PSTV are not inhibited by actinomycin D concentrations which reduce host 5S RNA synthesis by 80%. Thus, the primary role of actinomycin D in viroid synthesis may reflect either inhibition of a host-specified function or a general toxic effect on cell metabolism, and not evidence for the replication through a DNA intermediate.

Because actinomycin D produces toxic conditions in infiltrated tissue⁶ as well as protoplast⁷ systems, we have used a range of actinomycin D concentrations coupled with the monitoring of host nucleic acid as an internal control to produce a dose-response curve to evaluate actinomycin D sensitivity. Either foliar tissue of *Gynura aurantiaca* infected with CEV or potato tuber sprouts infected with PSTV were vacuum infiltrated with actinomycin D and ³²P in a Barth's solution (88 mM NaCl, 1 mM KCl, 0.83 mM MgSO₄, 0.41 mM CaCl₂ · 2H₂O, 0.34 mM Ca(NO₃)₂ · 4H₂O, 2.4 mM NaHCO₃, 1.5 mM Tris-HCl, pH 7.6) and incubated overnight before the extraction of nucleic acid by the phenol method¹². Using the parameter of incorporation of ³²P into DNA, it is possible to differentiate between sublethal doses of actinomycin D (0–10 μ g ml⁻¹) at which DNA synthesis is not affected and higher doses (10–40 μ g ml⁻¹) at which the inhibition of DNA synthesis may reflect general cellular impairment (Fig. 1). Incorporation of ³²P into total RNA decreased in a linear manner over the entire actinomycin D dose range.

RNA species were analysed by electrophoresis on 5% polyacrylamide vertical slab gel (Figs 2, 3)¹³. Samples were equalised by ³²P content so as to monitor only metabolically active cells

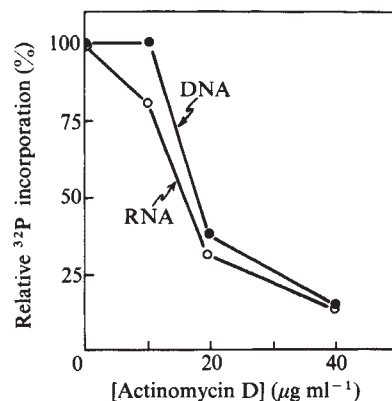


Fig. 1 Relative amounts of ³²P incorporation into RNA and DNA as determined by trichloroacetic acid (10% w/v)-precipitable radioactivity using equal nucleic acid concentrations. DNA radioactivity was determined from the acid-precipitable nucleic acids remaining after treatment with RNase A (Worthington, 200 units ml⁻¹ at 25°C for 45 min). RNA radioactivity was determined similarly after treatment with DNase I (Worthington, 20 μ g ml⁻¹ at 25°C for 15 min).

containing ³²P. The intensity of the RNA bands on the autoradiograph was quantitated by direct scanning of the X-ray film at 600 nm.

As purification of the viroid RNA involves partitioning into the 2 M LiCl-soluble fraction, the most appropriate internal control was the 5S ribosomal RNA. The synthesis of this low molecular weight (~36,000) species is significantly inhibited (Fig. 4) over the actinomycin D concentration range. The higher molecular weight ribosomal RNAs purified in the 2 M LiCl precipitate were similarly inhibited by actinomycin D. These results agree with the classical mode of action of actinomycin D as an inhibitor of DNA-directed RNA synthesis.

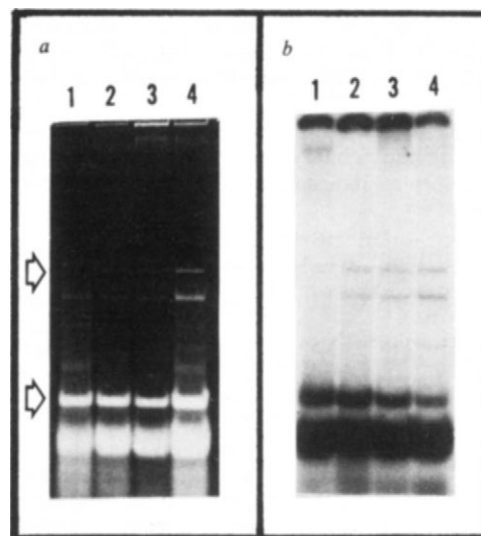


Fig. 2 Polyacrylamide slab gel (5%) electrophoretic patterns of 2 M LiCl-soluble nucleic acids obtained after ³²P incorporation into *Gynura* leaf tissue as described in the text. *a*, Nucleic acid patterns after staining with ethidium bromide (0.1 μ g ml⁻¹) in healthy tissue (1), and CEV-infected tissue without addition of actinomycin D (2), with 10 μ g ml⁻¹ actinomycin D (3), or with 20 μ g ml⁻¹ actinomycin D (4). *b*, Autoradiograph of the same gel after exposing Kodak XR-5 film for 7 days. All nucleic acids were treated with DNase I (Worthington, 2 μ g ml⁻¹ for 5 min at 25°C) just before loading on to the gel. The arrows indicate positions of CEV RNA (top) and 5S ribosomal RNA (bottom).

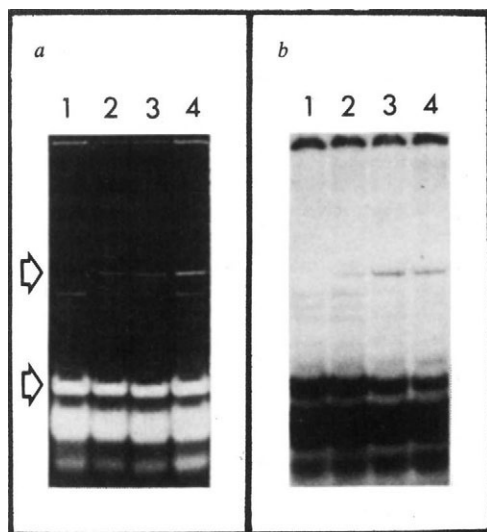


Fig. 3 Polyacrylamide slab gel (5%) electrophoretic patterns of 2 M LiCl-soluble nucleic acids obtained after ^{32}P incorporation into potato sprouts as described in the text. *a*, Nucleic acid patterns after staining with ethidium bromide in healthy potato sprouts (1), PSTV-infected potato sprouts without addition of actinomycin D (2), with $10\text{ }\mu\text{g ml}^{-1}$ actinomycin D (3), or with $20\text{ }\mu\text{g ml}^{-1}$ actinomycin D (4). *b*, Autoradiograph of the same gel. The nucleic acids were treated with DNase I as described in Fig. 2 legend. The arrows indicate positions of PSTV RNA (top) and 5S ribosomal RNA (bottom).

In contrast to the inhibition of host RNA synthesis, the synthesis of CEV in *Gynura aurantiaca* and potato spindle tuber viroid in potato (Figs 2, 3) was not as affected by actinomycin D. Therefore, it is not possible to confirm the previously reported actinomycin D inhibition of PSTV^{5,6} and cucumber pale fruit viroid⁷ in tomato. Because, in these systems, relatively high concentrations of actinomycin D were used to reduce total RNA synthesis by 80–90%, the interpreted inhibition may have resulted from a general toxic effect on cell metabolism and not the specific action of actinomycin D. Furthermore, the autoradiography procedure used here for analysing the synthetic rate of RNA species is more sensitive than slicing of cylindrical gels.

The absence of any significant inhibition of viroid synthesis is consistent with the primary role of the CEV complementary RNA sequences as intermediates in viroid replication³. Any

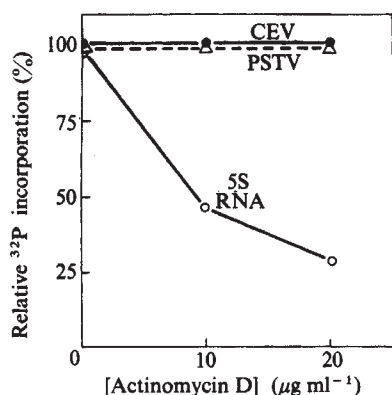


Fig. 4 Incorporation of ^{32}P into viroid RNA (CEV, PSTV) and 5S ribosomal RNA as influenced by actinomycin D concentration. The intensity of the radioactive RNA bands observed in the autoradiograph of the 5% polyacrylamide gel were quantitated by scanning the film at 600 nm and determining the area of each peak. The concentration of the radioactive viroid RNAs were set at 100% and the radioactive 5S RNA concentration determined accordingly.

inhibition of viroid replication by actinomycin D would seem to be mediated through a host-genome specified function and not through a viroid complementary DNA sequence. This inhibition by actinomycin D may be analogous to the sensitivity of viral RNA-directed RNA synthesis during early phases of infection¹⁴. Complementary RNA sequences in the PSTV system have been detected, whereas confirmation for the existence of complementary DNA sequences could not be accomplished (M. Zaitlin, unpublished). These data are consistent with the present finding that viroid synthesis is not specifically inhibited by actinomycin D. Data suggesting the absence of inhibition by actinomycin D have been confirmed by T. J. Morris (unpublished) using a different technical approach with the PSTV system.

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Immune and virus-induced interferons may activate cells by different derepressional mechanisms

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Virus-induced interferon (VIF), and immune interferon (IIF) produced by lymphocytes following activation by various mitogens (phytohaemagglutinin, concanavalin A, staphylococcal enterotoxin A) or by specific antigenic stimulation (tuberculin, bacterial toxoids, and viral antigens) show important functional differences. These include different antitumour¹ and immunoregulatory activities² and differential inhibition of activity by mercaptoethanol³. Another important difference has recently been shown: cells treated with IIF acquire the antiviral state much more slowly than those treated with VIF⁴. This may be due to (1) difference in cellular receptors, (2) presence of an inhibitor of antiviral activity in the IIF preparations, or (3) a different mechanism of activation of the antiviral state by the two types of interferon. The first two hypotheses seem unlikely, since (1) no difference between cell association by the two types of interferon has been detected⁴, and (2) the presence of the slow acting IIF preparation did not inhibit the rapid activation by VIF⁴. The present study shows that the different kinetics of induction of the antiviral state result from a major difference in the mechanism by which IIF and VIF activate cells.

It has generally been accepted that interferon itself is not the direct inhibitor of viral replication⁵. Instead, interferon acts by inducing the cells to produce one or more secondary protein(s), the so called antiviral or effector protein(s), which are needed for ultimate antiviral activity by cells. In support of this model one laboratory has reported that at least one newly formed protein correlates with the antiviral activity of VIF-treated cells⁶. It has also been shown, by using metabolic inhibitors, that VIF directly induces the production of the antiviral protein(s) without first

Table 1 Effect of cycloheximide on development of the antiviral state by virus-type and immune interferons

Type of interferon*	Cells	Cycloheximide added	log ₁₀ virus HA yield†
None	Mouse L	No	2.1
		Yes	2.1
Mouse, virus-induced	Mouse L	No	<0.3
		Yes	<0.3
Mouse, immune	Mouse L	No	<0.3
		Yes	1.5
None	Human WISH	No	1.8
		Yes	1.8
Human, virus-induced	Human WISH	No	<0.3
		Yes	<0.3
Human, immune	Human WISH	No	<0.3
		Yes	1.5
None	Human foreskin	No	1.8
		Yes	1.8
Human, virus-induced	Human foreskin	No	<0.3
		Yes	<0.3
Human, immune	Human foreskin	No	<0.3
		Yes	1.8

Mouse virus-induced interferon was obtained from mouse C-243 cell cultures induced with Newcastle disease virus as previously described⁹. Human virus-induced interferon was obtained by the Antiviral Substances Program, NIAID and was produced as described previously¹⁰. Mouse and human immune IFs were obtained by stimulation of lymphoid cell cultures with staphylococcal enterotoxin A and were characterised as immune-type IFs as described previously¹¹.

* Interferon used at 100 reference units ml⁻¹.

† GD-7 virus haemagglutinin yield was used for measurement of resistance in mouse cells, and Sindbis virus haemagglutinin yield was used for human cells.

inducing intermediary proteins⁷. Specifically, mammalian cells treated with VIF in the presence of cycloheximide developed the antiviral state immediately after reversal of the drug action. This finding indicates that an mRNA leading to the production of the antiviral protein was transcribed in the presence of VIF and cycloheximide and was translated only after reversal of cycloheximide action⁷. The addition of actinomycin D before removal of cycloheximide did not block the development of viral resistance. This suggests that VIF directly induces the antiviral protein without the need of intermediary proteins.

The same experimental plan was used to study the mechanism of activation of the antiviral state by IIF. In a first set of experiments, cultures of murine or human cells were treated with cycloheximide and then exposed to 100 reference units of homologous VIF or IIF, as previously described⁸. In our assay the titres reported as reference units correspond exactly to the actual titres observed in the experiments after 24 h incubation of interferon with the cells. The cultures were washed free of interferon and cycloheximide 4–5 h later, and challenged with GD-7 virus (mouse cells) or Sindbis virus (human cells). The virus yield was measured after 18 h incubation. The results are shown in Table 1.

As has been shown previously, cycloheximide did not prevent development of the antiviral state in mouse or human cells treated with VIF, suggesting that the mRNA for the effector protein(s) was induced by VIF during inhibition of protein synthesis and was rapidly translated after removal of cycloheximide. In contrast, cells treated with human or mouse IIF in the presence of cycloheximide failed to develop a significant level of antiviral activity. This finding might indicate that cells treated with IIF in the presence of cycloheximide either did not

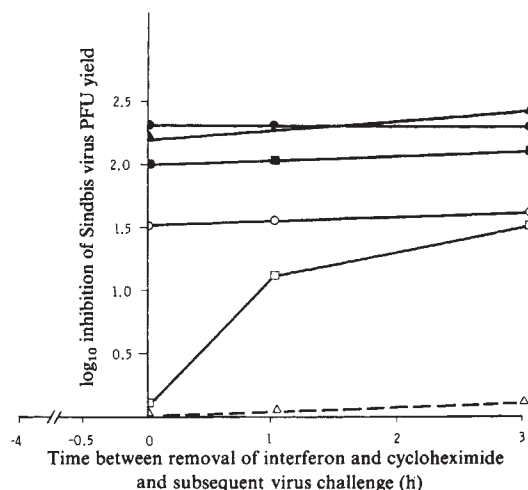


Fig. 1 Development of the antiviral state in human foreskin fibroblast cultures challenged at various times after exposure to human leukocyte interferon (VIF) or immune interferon (IIF) in the presence or absence of metabolic inhibition of protein or RNA synthesis. Four sets of cultures (six tubes per set) were pretreated with cycloheximide, 20 µg ml⁻¹, and 15 min later, 100 reference units of VIF (●) or IIF (○) were added to each tube. At the same time VIF (●) or IIF (○) was added to other cultures not treated with cycloheximide. After 3.5 h of incubation at 37 °C, actinomycin D, 5 µg ml⁻¹, was added to two of the sets of cultures treated with cycloheximide and IF (▲ and △). After 30 min, IF and the inhibitors were removed by multiple washing and the cultures were challenged thereafter at the indicated times with Sindbis virus. The level of the antiviral state was measured by determining the inhibition of virus yield in a single growth cycle as described previously⁹. Control cultures treated with the metabolic inhibitors as indicated above showed that protein synthesis (as measured by incorporation of ¹⁴C-leucine) was inhibited by 95% but RNA synthesis (as measured by incorporation of ³H-uridine) was unaffected during treatment with cycloheximide. After removal of cycloheximide, protein synthesis resumed fully within 0.5 h, indicating the reversibility of the inhibitor. In comparison, transcription was irreversibly blocked (92%) by the added actinomycin D. Additional control experiments showed that: (1) cell cultures treated with metabolic inhibitors for the times indicated above gave full yield of virus after removal of the drugs, and (2) treatment of the cells with 5 µg ml⁻¹ of actinomycin D for 30 min before addition of IF was completely able to prevent the development of the antiviral state. During the experiments the incubation temperature was carefully maintained at 37 °C as described previously⁸.

produce mRNA for the effector protein(s) or else they did produce this mRNA for the antiviral protein(s) but it was not translated quickly enough to prevent replication of the challenge viruses. To test these hypotheses, two-stage experiments were designed. During the first stage, production of mRNAs was permitted to occur by treating cells with VIF or IIF during inhibition of protein synthesis by cycloheximide, while in the second stage these mRNAs were allowed to be translated after addition of actinomycin D to block further RNA transcription. Specifically, cell cultures were pretreated with cycloheximide for 15 min and then treated with VIF or IIF in the continued presence of cycloheximide. After 3 or 5 h actinomycin D was added to some of the cultures to inhibit any further transcription of mRNA and 30 min later the cultures were washed three times (to remove interferon and reverse the inhibition of protein synthesis by cycloheximide but not the inhibition of transcription of RNA by actinomycin D). Some of the cultures were then challenged immediately, while others were challenged 1 and 3 h later. The results of a representative experiment are shown in Fig. 1.

It may be seen that, as previously observed⁷, cultures treated with VIF developed maximal resistance when challenged immediately after removal of interferon, regardless of the

presence of cycloheximide and the subsequent addition of actinomycin D. This result confirms the direct induction of the effector protein(s) by VIF. In contrast, cultures treated with IIF in the presence of cycloheximide alone failed to show detectable resistance when challenged with virus immediately after removal of the inhibitor, but gradually acquired the antiviral state when challenged 1 and 3 h later. The somewhat lower level of antiviral activity which appears to be induced by IIF as compared to VIF in Fig. 1 is due to the slower kinetics of cell activation by IIF (ref. 4) since the same activity was induced by both IFs at 24 h. However, again in contrast to the results with VIF, if actinomycin D was added before removal of cycloheximide, no detectable resistance appeared. The same results were obtained in mouse L cells treated with mouse VIF or IIF or when 30 µg per ml of cordycepin or 1-β-ribofuranosylbenzimidazole (DRB) were used as inhibitors of mRNA synthesis instead of actinomycin D, or when larger amounts of interferon (500 units instead of 100) were used. These findings rule out the delayed translation hypothesis and suggest that in the presence of cycloheximide IIF does not directly induce the formation of mRNA for the effector protein(s).

To further exclude the possibility that the IIF-induced mRNA for the antiviral protein(s) is translated more slowly than that induced by VIF, mRNA was induced in cells treated with IIF alone. Its rate of translation was then determined by blocking further mRNA synthesis with actinomycin D and thereafter measuring the level of viral resistance every hour. The data showed that cells stimulated with IIF, treated with actinomycin D after 3.5 h, and then challenged immediately or every hour thereafter for 4 h developed 1.5 log₁₀ of viral inhibition prior to the addition of actinomycin D; however the level of resistance did not rise thereafter, indicating that, similar to VIF, there is no delayed translation of the mRNA for the effector protein(s).

Another, though unlikely, possibility is that IIF simply induces a less stable mRNA for the antiviral protein. If this were the case, then the development of resistance in cells which would have produced the putative unstable mRNA after treatment with IIF and cycloheximide would begin at the same time as in cells containing stable, VIF-induced, mRNA but the maximum level of resistance would be lower. Instead, the first development of resistance is late and the maximum resistance is the same (Fig. 1). There are other more complex possible explanations, considering the multiple variables, but it is clear that IIF represents a novel mechanism, requiring a more complex cell-activating process than does VIF.

Taken together, the findings imply that there are multiple genetic derepressional events in the mechanism by which IIF activates the antiviral state. This interpretation is necessarily indirect due to the use of metabolic inhibitors as probes; however the conclusion is relatively strongly supported since the specificity of action of actinomycin D, cordycepin and DRB has been reasonably well established. Therefore the most probable interpretations are that VIF directly induces a mRNA for the antiviral or effector protein(s)⁷, but IIF does not directly induce functional mRNA for the effector protein(s). Studies are in progress to identify the product(s) of the gene(s) activated by IIF.

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Thymic nurse cells—Ia-bearing epithelium involved in T-lymphocyte differentiation?

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The vertebrate thymus has a crucial role in the development and organisation of the immune system. Physiologically, maturation of primitive incompetent precursor cells to immunocompetent T lymphocytes takes place in the thymus¹. Recently, evidence has been presented suggesting that the capacity to recognise determinants of the self major histocompatibility complex (MHC), which underlies MHC restriction of T-lymphocyte recognition, is also acquired within the thymus². Pathologically, T-cell lymphomas are known to arise within the thymus³. In all three cases, it is probable that at least some developmental events require close spatial interactions, if not direct contact, between migrating lymphoid cells and sessile stromal thymus elements. We describe here a state of maximal contact between both thymic cell partners, which might represent certain contact-dependent stages of T-cell development. We found that in all normal mouse and rat strains tested so far, the thymuses contain a sizeable number of huge cells of epithelial origin, which engulf and release up to 50 thymic lymphocytes. As the epithelial cells do not degrade the invading lymphocytes, but may rather provide microenvironmental requirements necessary for lymphocyte proliferation and differentiation, we call them 'thymic nurse cells' (TNCs). These TNCs express all the MHC determinants required by a cell involved in the establishment of T-lymphocyte MHC restriction. They express high doses of K/D antigens of the H-2 complex, and equally high doses of I-A region determinants. However, TNCs lack surface immunoglobulin and T-lymphocyte-specific Thy-1 antigen.

We have already developed a method that allows culture of rodent thymus stromal cells as pure monolayers⁴. This method is based on tryptic dissociation of minced thymic tissue by a stepwise digestion procedure. After repeated, exhaustive trypsinisation of thymic material, we recently found that unusually large, intact round cells (diameters up to 50 µm) can be recovered from all preparations. These round cells, TNCs, contain high numbers of small to medium-sized lymphoid thymocytes. Due to the enormous size of these cells, it is possible to isolate them from other, smaller lymphoid or stromal elements by simple repeated sedimentation through fetal calf serum layers. We recover routinely about 15 × 10³ TNCs from one young adult mouse thymus and up to 10 × 10⁴ TNCs from corresponding rat organs.

A number of observations suggest that TNCs are epithelial cells rather than macrophages, and that the engulfed lymphocytes have actively entered the TNCs rather than being phagocytosed. The crucial morphological features of TNCs revealing their epithelial nature are demonstrated in Fig. 1. This ultra-thin section shows a typical TNC incorporating at least seven thymocytes. The well organised tonofilament bundles in the TNC cytoplasm, and the typical vacuolisations make them indistinguishable from the cortical epithelial cells described *in situ*⁵. TNCs also functionally behave like epithelial cells. They are released from the thymic stroma only after extensive trypsinisation. They thus must be firmly anchored within the stromal network. This is in contrast to macrophages, which can be released by mere mechanical dissociation. When taken into culture, TNCs do not promptly adhere to plastic surfaces, as macrophages do, but rather tend to form cell aggregates. Finally, long-term cultures of purified TNC fractions display classical epithelial morphology lacking both mesenchymal and macrophage components, which may predominate in infranucleated thymus cultures.

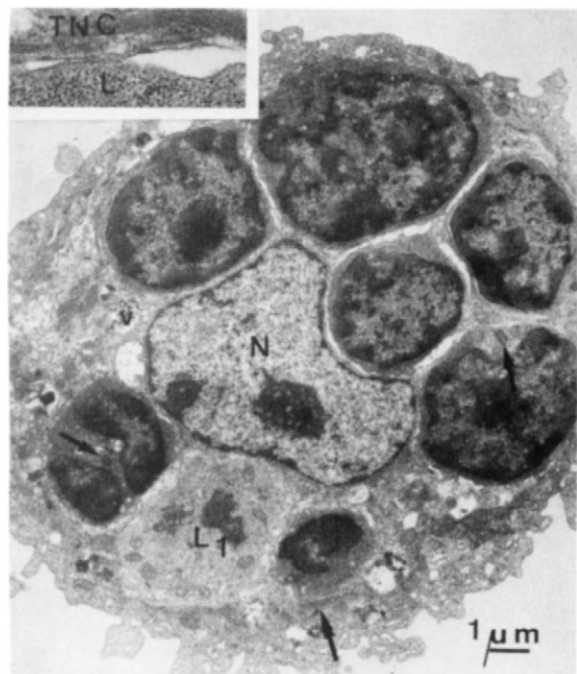


Fig. 1 Thymic nurse cell containing intact, proliferating thymocytes. Mitotic thymocyte (L_1), and centrioles in thymocyte cytoplasm (arrows). TNC nucleus (N) with typical prominent, reticular nucleolus. Prominent features of the TNC cytoplasm: vacuoles filled with coarse, granular material (v), and tonofilament streams in the periphery (double arrow). Inset: higher magnification of contact area between thymic lymphocyte (L) and TNC-containing tonofilaments. Thymuses from 40 young adult female C3H/f mice were washed in cold phosphate-buffered saline (PBS), minced with scissors, and subjected to differential trypsinisation⁴. The minced tissue was transferred into a trypsinisation bottle, and agitated in cold PBS for 5 min to remove most of the loose thymocytes and macrophages. The remaining tissue fragments were softened by further agitation in warm trypsin/DNase solution (50 ml 0.25% trypsin, plus 0.02 mg ml⁻¹ DNase in Ca²⁺- and Mg²⁺-free PBS) for 10 min. After discarding the supernatants, the main trypsinisation rounds were carried out in warm trypsin/DNase solution for 20 min per round. Single cell supernatants containing appreciable numbers of TNCs were pooled and resuspended in 3 ml HEPES-buffered Eagle's medium (EM-H). This suspension was layered on top of 10 ml undiluted, heat-inactivated fetal calf serum (FCS) in a conical 40-ml glass centrifuge tube. After sedimentation at unit gravity for 15 min, the top fraction containing the majority of all small cells was discarded, whereas the serum fraction and the sediment containing the large TNCs were collected. The cells were resuspended in 0.5 ml EM-H, layered over 2 ml FCS in a 10-ml Falcon plastic tube, to purify further the TNC populations. Practically pure TNC fractions were obtained after 4 or 5 sedimentation rounds. For electron microscopy TNC fractions were fixed in 3% glutaraldehyde, postfixed in 1% OsO₄, embedded in Epon before ultra-thin sections

The majority of the thymocytes engulfed in TNCs appear to be fully intact, as judged by morphological criteria. Many of these thymocytes seem to be involved in proliferation, as suggested by the unusually high incidence of mitotic figures and centrioles (Fig. 1). The thymocytes are completely enclosed by a TNC membrane, but we never observed signs of fusion between both cell partners. That the surrounding caveolar cleft does not communicate with the extracellular space is indicated by the finding that antibodies binding to thymocytes, for example anti-Thy-1 antibodies, do not reach the engulfed thymocytes. Our observation that initially pure TNC fractions cultured overnight contain high numbers of viable free thymocytes suggests that at least a major proportion of the internalised thymocytes can migrate out of the TNCs.

The phenomenon that migratory cells can invade larger, sessile cells without being digested, has been termed 'emperipolesis'⁶, and was mainly described in tissue culture

conditions. Note, however, that *in situ* emperipolesis of thymocytes in thymic stroma cells has been observed in normal rat thymuses⁷, as well as in thymuses from mice undergoing graft-versus-host disease⁸. It seems reasonable to assume that migration of thymocytes into autochthonous stromal cells is initiated by recognition of stromal determinants by the migrating thymocytes. Indeed it has been shown⁹ that normal thymocyte populations contain considerable numbers of clones capable of recognising self determinants expressed on autochthonous stroma cells.

It is tempting to speculate that the TNC phenomenon has some role in intrathymic T-lymphocyte differentiation, and, in particular, in the T-cell education/selection process, as recently proposed by Zinkernagel¹⁰ and Bevan¹¹. These authors found that the capacity of the T-cell compartment to acquire self-MHC-restricted reactivity against foreign determinants, which may coincide with generation of T-cell diversity¹², is developed within the thymus. T-lymphocyte reactivity is known to be restricted by self determinants coded for both by K/D as well as I regions of the murine MHC. If selection processes within the thymus would involve TNCs, both K/D and I region determinants should be expressed on TNC membranes.

We determined the H-2 antigen formula of TNC membranes using indirect immunofluorescence and monoclonal antibodies, which are directed against determinants of various H-2 subregions (Table 1). TNCs which do not bind anti-Ig and anti-Thy-1.2 antibodies express high doses of classical K/D region histocompatibility antigens on their surface, and equally high concentrations of I-A region determinants. The latter finding is particularly remarkable, as I region antigens are known to be restricted to relatively few cell types, such as (B) lymphocytes, macrophages and sperms¹³.

Some recent reports have demonstrated the presence of I-region antigens on thymic components. However, it has proved difficult to classify exactly the antigen-positive cell type using light microscopical screening of thymus sections^{14,15}. This holds true in particular for distinction between thymus epithelium and thymus macrophages, which may be abundant constituents of the organ¹⁶. We applied a fluorescence double labelling method, which allowed functional classification of macrophages, and, at the same time determination of the surface antigen phenotype. Cellular phagocytosis was determined using TRITC-labelled zymosan, whereas the membrane antigens were assayed by indirect immunofluorescence using monoclonal antibodies and FITC-RAMIG, as described in Table 1. Figure 2 shows a mixed T-cell population composed of TNCs, macrophages and a few thymocytes, which were first exposed to

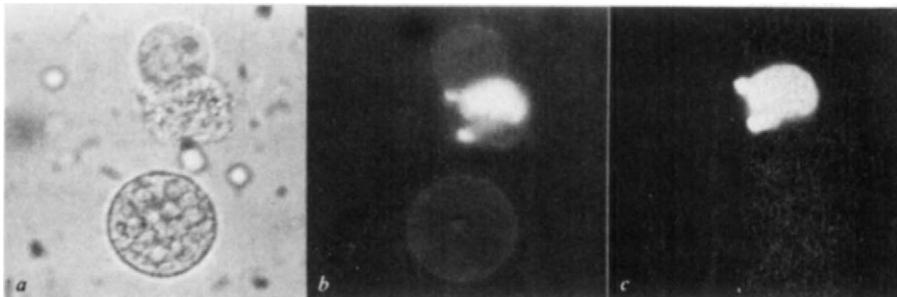
Table 1 Antigens expressed on TNC membranes

Antisera (batch code)*	C3H/f (H-2 ^k)		C57BL/6 (H-2 ^b)	
	TNC†	Thymocytes	TNC	Thymocytes
FITCS-RAMIG alone	—	—	—	—
+ anti-Thy-1.2 (F7D5)	—	++	—	++
+ anti-K ^k (H100-27R9)	++	±	±	—
+ anti-I-A ^k (H118-49)	++	—	±	—
+ anti-I-A ^b (B17-123.R4)	±	—	++	—
+ anti-H-2D ^b (B22-277.R1)	—	—	++	±

* Monoclonal hybridoma antibodies were derived from hybridoma-induced ascitic fluid¹⁷. Anti-Thy-1.2 (F7D5) was from Olac. FITC-RAMIG was a fluorescein-labelled rabbit anti-mouse immunoglobulin antibody preparation from Miles-Yeda.

† Cell preparation and labelling procedures as in Fig. 1 legend. The mice were 4–6-week-old females. Fluorescence was scored: ++ bright, distinct ring-shaped fluorescence; + definite, though less intensive ring shaped; ± vague fluorescence, without ring formation, and — no fluorescence at all.

Fig. 2 Expression of Ia antigens on TNCs and thymic macrophages. *a*, Light microscopy of a semi-purified TNC fraction prepared from C3H/f thymuses following the methods described in Fig. 1. TNC (lower cell), thymic macrophage (centre), and unclassified thymic stromal cell (top), along with three thymocytes. *c*, TRITC-labelled zymosan ingested by a thymic macrophage, viewed under red-pass filter. Zymosan (20 mg, lot no. DZ-3185, Schwarz/Mann) was suspended in 30 ml boiling 0.5 M sodium bicarbonate solution (pH 9.5). Total de-aggregation of the particles was achieved by additional short ultrasonication. After cooling the suspension, we added 1.6 mg tetramethyl-rhodamine-isothiocyanate, isomer R (TRITC, Nordic Laboratories). The mixture was stirred overnight at 4 °C. The stained zymosan particles were centrifuged (2,000g, 10 min, 4 °C) and washed five times in each 30 ml ice cold acetone water (3 parts distilled water, 1 part acetone), before being resuspended in 1 ml 0.5 sodium bicarbonate solution. This stock solution was stored in the dark at 4 °C. For the phagocytosis assay, TNC populations were resuspended in 1 ml EM-H, supplemented with 10% FCS (1×10^6 cells ml^{-1}). We added 20 μl TRITC-zymosan stock solution. After 30 min incubation at 37 °C in normal atmosphere, the cells were centrifuged and resuspended in 0.5 ml EM-H, and underlayered with 2 ml FCS. Within 15 min, most of the living thymus cells sedimented to the bottom, whereas the small free zymosan particles were left in the upper layer. *b*, Membrane fluorescence of the same cells under green-pass filter. For immunofluorescence demonstration of Ia antigens, TNC populations were resuspended in Eppendorf conical plastic tubes, in 100 μl PBS (plus 0.2% NaN_3). We added 10 μl undiluted monoclonal antibody containing ascites fluid H-118-49 (reacting against Ia.2, I-A^k, ref. 17). After 30 min incubation on ice, the cells were washed twice in each 1 ml PBS, resuspended in 200 μl PBS. After addition of 20 μl fluorescein-labelled rabbit anti-mouse immunoglobulin antibody solution (diluted 1:6 in PBS, Miles-Yeda), cells were incubated on ice for a further 30 min, washed twice, and screened under a Zeiss fluorescence microscope. Photomicrographs were taken on Kodak Tri-X pan film (400 ASA), with an exposure of 60 min. The macrophage ingested zymosan shows some residual fluorescence in these conditions.



TRITC-Zy and subsequently to anti-I-A antibody and FITC-RAMIG. Both macrophages and TNCs, but no free thymocytes, express I-A region determinants, as indicated by the fluorescence under a green-pass filter (Fig. 2*b*). Our investigations showed, moreover, that TRITC-zymosan was never ingested by TNCs, and also that TRITC-zymosan ingesting cells, macrophages, never contained more than one morphologically intact lymphocyte (Fig. 2*c*). This provides further support for our previous ultrastructural evidence that TNCs and macrophages are distinct cell types.

Thus, TNCs indeed possess all antigenic requirements needed by a thymic stromal cell involved in a selection process of self-restricted T-lymphocyte clones. We are attracted by the possibility that immature T-cell progenitors with receptors for self antigens recognise self-MHC determinants on TNC membranes as a first step of selection. The recognising cells could be caused to invade the TNC, and, within the peculiar intracellular environment, could be induced to differentiate to immunocompetent T cells. The unusually high rate of proliferative activity of the engulfed thymocytes seems to support such a hypothesis.

Our data at this stage are merely descriptive and do not relate to actual functions of TNCs in T-cell maturation. Our techniques to isolate practically pure TNC populations from normal thymuses might, however, form a good basis for future experiments.

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Production of alloreactive T-cell lymphomas

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Experiments predicting^{1,2} or demonstrating lymphomagenesis in mice undergoing graft-versus-host (G-v-H) or chronic immunological reactions have been reported^{3–5}. In such cases, lymphomagenesis seemed to operate through the agency of activated murine leukaemia viruses (MuLV) and occurred only after very long latent periods, and most (but not all) lymphomas were of host origin⁴. No attempts were made in these studies to assess the relative role of T-cell subpopulations in lymphomagenesis. We have proposed elsewhere that certain MuLV-induced T lymphomas might be the progeny of that subset of normal thymocytes bearing antigen specific T-cell receptors for MuLV envelope antigens^{6–10}. Further, the MuLV binding to these cells provides both a portal of infection and a mitogenic signal^{6,9,10}. These cells and their thymic progeny would be subject to continuous restimulation by the endogenously produced MuLV envelope glycoproteins, and thus the apparent and 'uncontrolled' proliferation of the cells would be due to their inability to resolve the viral infection and escape the resultant antigenic microenvironment. This receptor-mediated leukaemogenesis hypothesis predicts that any T-cell clone that cannot escape its stimulating antigens would appear to be lymphomagenic in that environment. Recent experiments in our laboratory have demonstrated that unique clones of strain A alloreactive T cells exist which can recognise hybrid mixed lymphocyte reaction (MLR)-stimulating determinants present on (C57B16 × A/J)F₁ (B6A) stimulator spleen cells^{11–15}. A variety of such clones have been isolated and characterised^{13–15}. We reasoned that, using these clones, it might be possible to generate alloreactive T-cell lymphomas by *in vivo* injection of these cells into hybrid B6A mice. We report our successful results here.

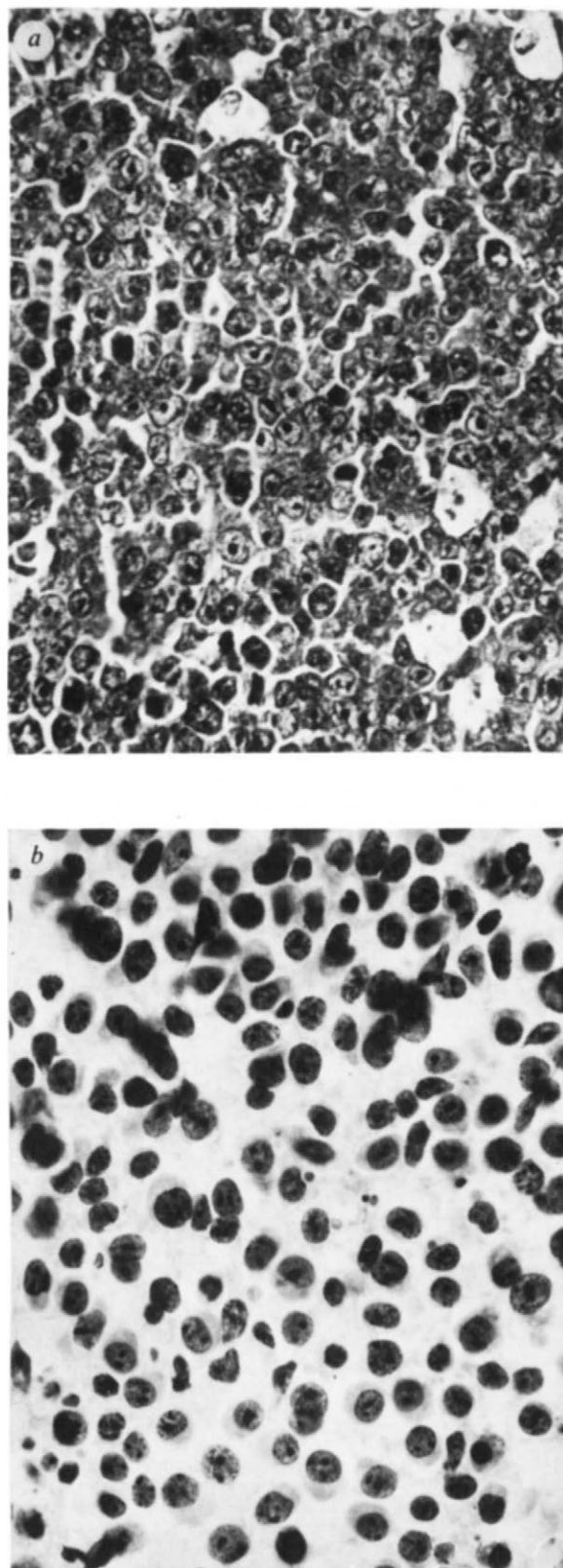


Fig. 1 *a*, Light microscopic appearance of immunoblastic lymphoma involving pancreatic lymph node (initial tumour). *b*, Cellular imprint preparation from pancreatic lymph node demonstrating immunoblastic morphological features of tumour cells: large central nucleoli, thick nuclear membranes and strongly staining cytoplasm (alcohol fixation, haematoxylin-eosin stain, $\times 640$).

Table 1 Proliferative responses of clone A(B6A)1-1

A	Stimulator cells			
	B6	B6A	DBA/2	SJL
2,105 ± 198	2,865 ± 111	55,531 ± 851	3,104 ± 144	1,832 ± 87

The response ($\pm$ s.d.) of triplicate cultures of 25×10^3 A(B6A)1-1 cells measured as c.p.m. of an overnight pulse with $2 \mu\text{Ci}$ of ^3H -thymidine following 48 h co-culture with 1×10^6 irradiated (3,300 R) spleen cells (stimulator cells) of strains A/J(A), C57B16 (B6), (B6A)F₁, DBA/2 and SJL/J.

Studies by Cole and Nowell⁵ suggested that repeated and prolonged proliferative activity would increase the probability of transformation in stimulated parental strain lymphocytes in a parent-into-F₁ G-v-H system. The use of alloreactive T-cell clones as the source of donor lymphocytes generates a unique situation in which the host is incapable of rejecting the donor cells, and the donor cells are in a situation of continuous stimulation and proliferation. As this clone apparently contains only Ly-1⁺ 'helper' T cells (see below), induction of regulatory or cytotoxic T cells of donor origin would be unlikely and the donor cell proliferation would therefore be unregulated in B6A hosts.

The alloreactive T-cell clone A(B6A)1-1 selected for *in vivo* injection has been described previously¹³. Table 1 shows the reactivity pattern of clone A(B6A)1-1 on a small panel of stimulator cells. This clone has been carried for over 18 months *in vitro* without undergoing transformation and needs serial restimulation for propagation. At time 0, six separate mice, two each of strains A, B6 and B6A were injected intraperitoneally (i.p.) with 5×10^6 alloreactive T cells of this clone. One mouse from each of the three groups received sublethal (200 R) total body X-ray irradiation. Approximately 1 month after i.p. injection of clone A(B6A)1-1 cells, both hybrid B6A animals had large abdominal masses whereas neither the A nor the B6 animals showed any signs of tumour growth. Both B6A animals were killed and tumours, which seemed to be confined to the mesenteric lymph nodes, were excised and characterised as described below. The *in vivo* injected mice of strain A and B6 were killed 2 months after i.p. injection and none of the four mice showed evidence of tumour growth macroscopically, histologically or by attempted *in vitro* passage of lymphoid cells from their thymus, spleen or lymph nodes.

The lymphomas which arose in the two B6A mice were uniform in appearance. There was extensive replacement of lymph node elements by immunoblastic cellularity in mesenteric and pancreatic nodal tissues, and the spleen and pancreas were massively infiltrated by similar lymphoid cellularity (Fig. 1*a, b*). The immunohistological appearance of these lymphomas at the light and electron microscopic level will be reported elsewhere. The involved tissue was excised and the cells were passaged both *in vivo* and *in vitro*. Serological characterisation of the lymphomas for selected cell-surface determinants was carried out in the laboratories of Chella David at Mayo Clinic and Bonnie Matheson at NIH, by conventional techniques of complement-mediated cytotoxicity, indirect immunofluorescence and absorption. The lymphomas derived from both mice were serologically identical and all were of cell-surface phenotype H-2K^k, 1a^a, Ly-1.2 and Thy-1.2 positive. They were surface immunoglobulin, H-2K^b, H-2D^b, 1a^b and Ly-2 negative. Preliminary studies on the virological status of these lymphomas using indirect immunofluorescence have shown that they are surface gp70 positive and p30 negative.

Despite continuous growth *in vitro*, on three separate serial *in vivo* passages the lymphomas derived from the original i.p. injection of B6A mice with clone A(B6A)1-1 would only grow in B6A mice (with or without X-ray irradiation). Repeated efforts to passage these lines in animals of strain A and/or B6 (with or without irradiation) failed to yield any tumour growth. Surprisingly, the tumour passed and cloned *in vitro* grew as a

continuously replicating cell line. Thus, we have a perplexing situation which may be explained by two possibilities: either antigen drive is requisite for *in vivo* growth but not necessary for *in vitro* proliferation, or growth in B6A mice and the failure of these lymphomas to grow in strain A or B6 mice could be attributed to host regulation rather than lack of appropriate antigenic signal. This system supports the concept of lymphomagenesis occurring in a situation of antigen-driven lymphoproliferation. The model lends itself to extensive exploration as to the origin of the transforming event and the search for alloantigen receptors and/or cloned products on such alloreactive T cells, and has perhaps provided us with a useful tool for future hybridisation studies of immunocompetent T cells.

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Common pathways of interferon and hormonal action

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The action of interferon¹ as well as polypeptide hormones² has been shown to be transmissible between cells, possibly through gap-junctional transfer of secondary messenger molecules. This and other similarities between interferon and polypeptide hormones³ have led us to propose that there is a common cellular pathway of interferon and hormonal action. If correct, this hypothesis would predict that interferon should cause a species-specific hormonal response and a hormone should induce tissue-specific antiviral activity. If these two responses are mediated by similar secondary messengers, they should be transmissible and cross-activate cells. Here, we show that interferon caused a species-specific hormonal response (noradrenaline-like stimulation of the beat frequency of cultured mouse myocardial cells). Noradrenaline induced an interferon-like antiviral state in mouse myocardial cells but not human amnion (WISH) cells. In conditions which demonstrate interferon-induced transfer of viral resistance, exposure of co-cultures of mouse myocardial cells and WISH cells to either human interferon or noradrenaline caused an increased beat frequency in the myocardial cells and development of antiviral activity in WISH cells, respectively. These studies strongly suggest common pathways of interferon and hormonal stimulation that are transmissible between cells.

Figure 1a shows the dose response of the spontaneous beat frequency of cultured mouse myocardial cells to noradrenaline and is in agreement with previously published results^{2,4,5}. Increases in beat frequency also resulted from treatment of the cultures with mouse but not human interferon (Fig. 1a). The interferon concentrations required to cause a change in beat frequency are equivalent to those which induce antiviral activity and are well within the concentrations produced *in vivo*. The similarity of the slopes of the noradrenaline and interferon dose responses suggests that they increase the beat frequency by a similar mechanism. These results show that interferon can elicit a hormonal response in a species-specific manner.

Figure 1b shows that overnight treatment with noradrenaline caused a dose-dependent development of antiviral activity in myocardial cells. Interestingly, the maximal antiviral activity was observed at the same concentration (10^{-6} M) of noradrenaline as the maximal stimulation of beat frequency. This finding suggests a relationship between noradrenaline-induced increases in beat frequency and the development of antiviral activity. The noradrenaline-induced antiviral state was not as strong as that induced by interferon (0.7 to 1.0 log₁₀ reduction in virus yield compared with 2.0 log₁₀ reduction). As will be shown below, in identical conditions, noradrenaline did not cause antiviral activity in human amnion (WISH) cells, thereby demonstrating its tissue specificity. These findings demonstrate that in addition to its classical effect on beat frequency, noradrenaline treatment of its target (myocardial) cells causes the development of an antiviral state.

Table 1 shows that purified fibroblast interferon ($10^{8.5}$ units per mg protein) caused an increase in beat frequency. Additionally, the stimulatory effect of a crude interferon preparation was neutralised by specific anti-interferon antisera. In other studies, interferon preparations of varying purity increased the

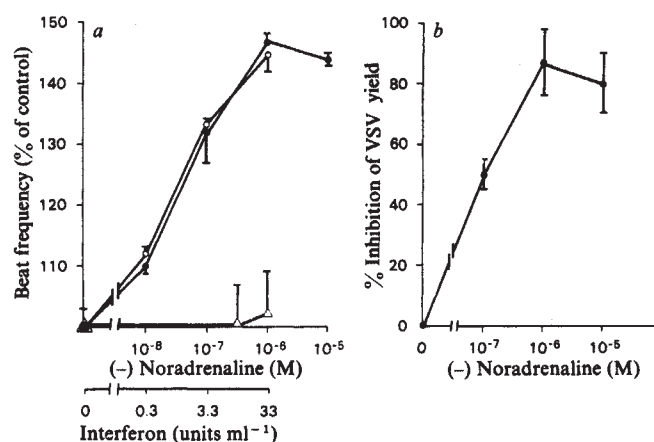


Fig. 1 Effect of (-)noradrenaline and interferon on the beat frequency (a) and virus replication (b) in cultured mouse myocardial cells. Except for the omission of filtering through nylon mesh, cells were prepared as previously described¹⁶. For the frequency experiments about 2.5×10^5 cells were plated into each well of Falcon multiwell tissue culture plates in 1 ml of Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 U ml⁻¹) and streptomycin (100 µg ml⁻¹). After 24 h cultures were placed at room temperature for 1 h to equilibrate and experiments were carried out at this temperature. During the experiments the medium remained near pH 7.4. Each value is the mean $\pm$ s.d. ($n = 4$ cells in parallel culture). Beat frequency was determined visually with the aid of a microscope ($\times 100$) and stopwatch (Scientific Products). Each cell served as its own control. Control basal counts were 60–80 beats per min. Beat frequencies were determined 5 min after the addition of 0.1 ml of the indicated concentrations of each compound at room temperature. For virus replication studies 2.5×10^4 cells were plated into each well of Falcon microtitre tissue culture plates in 0.1 ml of culture medium. After 24 h medium was replaced with the indicated concentrations of noradrenaline in 0.1 ml volume in duplicate cultures and incubated overnight at 37°C in 4% CO₂ atmosphere. Supernatant fluids were removed and cultures were infected with vesicular stomatitis virus (300 plaque forming units (PFU) per culture). Virus yields were determined 24 h later by a previously described microplaque assay¹. Control virus yields were about 1×10^6 PFU ml⁻¹. Each point represents the mean $\pm$ % inhibition of the control virus yield $\pm$ s.d. ($n = 4$). ●, (-)Noradrenaline; ○, mouse interferon; △, human interferon.

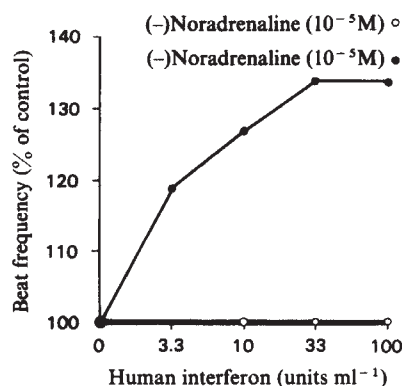


Fig. 2 Effect of human interferon on the beat frequency of mouse myocardial cells co-cultured with human amnion (WISH) cells. Mouse myocardial cells (2.5×10^5 per well) were plated into adjacent wells of a Falcon multiwell plate. Half of the wells then received 2.5×10^5 WISH cells. After overnight incubation, cultures were equilibrated to room temperature, human fibroblast interferon (1.8×10^6 units per mg protein) was added to achieve the indicated concentrations and the beat frequency ($n=3$) determined as described in Fig. 1 legend. After beat frequencies had been determined, (-)noradrenaline (10^{-5} M) was added to cultures which had been previously treated with human interferon ($100 \text{ units ml}^{-1}$) and the beats were again counted. ●, Myocardial cells + WISH cells; ○, myocardial cells.

beat frequency in direct proportion to their antiviral titre (data not shown). We also found (Table 1) that once myocardial cells were maximally stimulated with mouse interferon, addition of noradrenaline (10^{-5} M) caused no further significant increase. Also, interferon and noradrenaline increased the beat frequency with similar kinetics. Maximal stimulation with noradrenaline required about 1 min whereas interferon required 2 min (Table 1). This represents the most rapid reported final action of interferon. Taken together, these results strongly suggest that the effects of noradrenaline and interferon on the beat frequency of mouse myocardial cells occur through a common mechanism.

When the antiviral activity of noradrenaline was characterised, we found that the continued presence of noradrenaline and interferon was not required, as the antiviral state remained after overnight treatment and washing the cells to remove these substances. This shows that both interferon and noradrenaline act on the cell rather than on the virus. The interferon-induced antiviral state is inhibited by actinomycin D (refs 6, 7). Similarly, the induction of antiviral activity by noradrenaline was blocked by actinomycin D (Table 1). Like interferon, noradrenaline did not show virus-specific antiviral activity (see below and Fig. 3). In conditions of high multiplicity of infection, vaccinia virus is not sensitive to interferon¹ and was not inhibited by noradrenaline (data not shown).

The indication that interferon and noradrenaline share common pathways that may involve similar secondary messenger molecules implies that the hormonal activity of interferon and the antiviral activity of noradrenaline should be transmissible between cells. This hypothesis was tested with co-cultures of mouse myocardial cells and human amnion WISH cells. Human interferon did not increase the beat frequency of mouse myocardial cells (Figs 1a, 2). However, when human interferon was added to co-cultures of mouse myocardial cells and human WISH cells (which are sensitive to human interferon), there was a dose-dependent increase in the beat frequency of the myocardial cells. This effect was only observed when the myocardial cells were in contact with human cells. When noradrenaline (10^{-5} M) was added to co-cultures which had been maximally stimulated with human interferon there was no further increase in beat frequency, whereas noradrenaline addition to mouse myocardial cells treated with human interferon increased their beat frequency to that of the co-cultures (Fig. 2). The dose dependence of this response resembled that observed with mouse interferon on mouse myocardial cells (compare Figs 1a and 2). Both cell types in the co-cultures in the

presence of human interferon also developed antiviral resistance whereas human interferon-treated mouse myocardial cells alone did not. Ten units of human interferon caused a 90% reduction in vesicular stomatitis virus (VSV) yield from mouse myocardial cells co-cultured with human WISH cells. Kinetic studies showed that the increased beat frequency due to mouse interferon treatment of mouse myocardial cells precedes by 1–2 min that observed in co-cultures treated with human interferon (data not shown). The observed sequence is consistent with the initiation of events in the human cell by human interferon, and their subsequent transfer to the myocardial cell. These data show that in conditions which can lead to transfer of interferon-induced viral resistance, transfer of the hormonal activity of interferon occurs.

The possible transmissible nature of the antiviral activity of noradrenaline was explored by infection of co-cultures with poliovirus, which only infects human cells⁸. Figure 3 shows that noradrenaline did not induce antiviral activity in human WISH cells alone. However, it produced a dose-dependent decrease in poliovirus yield from the WISH cells in the co-cultures. Poliovirus did not replicate in mouse myocardial cells in the presence or absence of noradrenaline, and co-culturing myocardial cells and WISH cells did not affect the yield of poliovirus from the WISH cells. Similarly, mouse interferon (50 units ml^{-1}) treatment of co-cultures (1:1 ratio) of mouse myocardial cells and WISH cells resulted in a 85% reduction in poliovirus yield from the WISH cells (data not shown). When VSV was substituted for poliovirus, the yield of virus from either cell type alone or in combination was similar. Noradrenaline caused a dose-dependent decrease in VSV yield from myocardial cells alone (Fig. 1b), but had no effect on the virus yield from WISH cells alone (Fig. 3). As the cells were mixed in a 1:1 ratio, if there had been no development of viral resistance in the co-cultured WISH cells one would have expected about 50% of the yield of a co-culture without noradrenaline. Figure 3 shows that noradrenaline caused a dose-dependent decrease in the expected VSV yield, indicating antiviral activity in the co-cultured WISH cells. Thus, noradrenaline causes the transfer of nonspecific virus resistance between its target cell and other cells.

Table 1 Characteristics of the hormonal activity of interferon and the antiviral activity of (-)noradrenaline on mouse myocardial cells

Addition to mouse myocardial cells	Beat frequency (% of control)*	Time required for maximum change in beat frequency (min)†	% Inhibition of VSV yield‡
Purified interferon§ (50 units ml^{-1})	142 ± 8	2	ND
Purified interferon (50 units ml^{-1}) + (-)noradrenaline (10^{-5} M)	161 ± 14	ND	ND
Crude interferon (50 units ml^{-1})	151 ± 3	2	99 ± 1
Crude interferon (50 units ml^{-1}) + anti-interferon antisera (1:20 dilutions)	88 ± 18	>10	ND
(-)Noradrenaline (10^{-5} M)	ND	1	75 ± 3
(-)Noradrenaline (10^{-5} M) + actinomycin D (2 $\mu\text{g ml}^{-1}$)	ND	ND	0 ± 10

Procedures were as described in Fig. 1 legend. Crude interferon and anti-interferon antisera were mixed and incubated at room temperature for 30 min before addition to mouse myocardial cells. Beat frequency was counted. ND, not done.

* Values are means ± s.d.

† Times were derived from kinetics experiments as described in Fig. 3 legend.

‡ Procedures were as described in Fig. 1 legend. Noradrenaline and actinomycin D were added simultaneously. Values are means ± s.d.

§ $10^{8.5}$ Units per mg protein.

|| There was no change in beat frequency after 10 min.

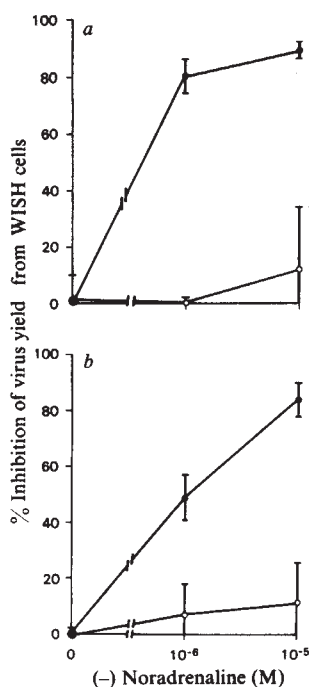


Fig. 3 Effect of (-)noradrenaline on virus replication in co-cultures of mouse myocardial cells and human amnion (WISH) cells. Mouse myocardial cells (3.0×10^4 per well) and human WISH cells (3.0×10^4 per well) were cultured alone or in combination (1:1 ratio) in Falcon microtitre tissue culture plates. After 24 h medium was replaced with the indicated concentrations of noradrenaline and incubated overnight. Supernatant fluids were removed and cultures were infected with 300 PFU of either VSV (a) or poliovirus (b). Virus yields were determined 24 h later by a microplaque assay¹. Each point represents the mean % inhibition of the control virus yield $\pm$ s.d. ($n = 6$). VSV yield from myocardial cells alone was inhibited (Fig. 1b). Poliovirus did not replicate in mouse myocardial cells. ●, Myocardial cells + WISH cells; ○, WISH cells.

Thus, we have demonstrated that interferon can have hormonal activity and that hormonal stimulation can result in interferon-type antiviral activity. From these findings we conclude that interferon and hormonal action are probably mediated by common pathway(s). The transmission of the reciprocal actions of interferon and noradrenaline not only gives further credence to a common pathway of their actions but also suggests that common transferred molecule(s) are generated after interaction of either substance with the appropriate cell membrane. Superficially, cyclic AMP seems a candidate for the interferon-induced increase in beat frequency, because cyclic AMP can cause this response^{2,9,10} and interferon in certain conditions can elevate cyclic AMP levels^{11,12}. However, cyclic AMP alone cannot account for the antiviral effects as it is not antiviral¹³ and interferon does not stimulate adenyl cyclase in all cells^{11,12}. A more likely situation is that cyclic AMP and/or another small molecule(s) is responsible.

A question which results from these studies is, is interferon a hormone? The many similarities between interferon and polypeptide hormones indicate that interferon should be classified as such, and this is supported by our inability to distinguish interferon action from a hormonal response. As such, the natural role of interferon may be regulatory, with its effects on virus infections being secondary. The instances of low levels of interferon in normal individuals may not result from inapparent virus infections but may reflect this more general interferon regulatory mechanism. Additionally, this could be related to the side effects observed during clinical trials using high levels of interferon¹⁴ as well as some aspects of viral pathogenesis. If interferon can cross-activate for other hormonal activities, this might explain the many diverse actions of interferon (for review see ref. 15). A shared mode of action of interferon and hormones would suggest that interferon action is not unique and makes

questionable the view that the varied biochemical changes in interferon-treated cells are interferon specific.

A second question is, what are the limits of responses to hormones? The present findings suggest that hormones may, in addition to their known actions, protect tissues against viruses or maintain differentiation. If this could be documented *in vivo*, a new strategy of tissue-targeted antiviral and antitumour therapy might evolve.

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Corrigendum

In the letter 'Gravitational magnetism' by S.-P. Sirag, *Nature* **278**, 535 (1978), in Table 1 the maximum magnetic moment, P , for Her X-1 should be 35×10^{26} A m².

Errata

In the letter by C. D. B. Bridges and S.-L. Fong, *Nature* **282**, 513 (1979), the title should have read: 'Different distribution of receptors for peanut and ricin agglutinins between inner and outer segments of rod cells'.

In the letter 'Oxidant damage mediates variant red cell resistance to malaria' by M. J. Friedman, *Nature* **280**, 245 (1979), the second sentence of Fig. 2 legend should read: 'The interaction of H₂O₂ and Hb results . . . '.

In the letter 'Inhibition of mixed lymphocyte response by monoclonal antibody specific for a rat T lymphocyte subset' by M. Webb *et al.*, *Nature* **282**, 841 (1979), the symbols in Fig. 1 have been wrongly given. The correct designation is: —●— w3/25 IgG, —○— W3/25 F(ab')₂, —□— W3/13 IgG.

In the News and Views article 'Is anyone out there?' *Nature* **281**, 528 (1979), two lines were inadvertently omitted leading to the attribution of views to I. Shklovsky entirely opposite to those he actually stated. The correct sentence should read: "I. Shklovsky of the Sternberg Astronomical Institute suggested that the choice of a value for N is a function of the age of the investigator. He attributed 'youthful optimism' to T. Kuiper of the Jet Propulsion Laboratory who suggested that N may be a very large number ($\geq 10^9$).

MATTERS ARISING

Reevaluation of mitosis in the red alga *Porphyridium purpureum*

BRONCHART AND DEMOULIN have reported that mitosis in the red alga *Porphyridium purpureum* is 'unusual', with no kinetochores or well defined spindle pole bodies (SPBs), and they described a unique association of microbodies (MBs) with the spindle poles¹. Our studies, however, lead to different conclusions and we report here some of our results.

P. purpureum, isolate 637 of the University of Texas Culture Collection of Algae, was grown in von Stosch enriched seawater medium under a 14 h light/10 h dark photo regime in 60-mm Petri dishes. Light microscopy showed 30% of the cells dividing 11 h after the start of the light period. Cells were fixed *in situ* at that time in culture media-phosphate-buffered 1% glutaraldehyde, postfixed in osmium, stained *en bloc* with 2% uranyl acetate, dehydrated in acetone, embedded in Epon, sectioned and poststained with lead.

Electron microscopy of serial sections showed that most dividing cells were in prophase. Each cell contained a large, stellate chloroplast and an eccentric nucleus. In early prophase a pair of SPBs was associated with the nuclear envelope (NE) in a region destined to become one of the division poles. Each SPB (Fig. 1a, b) had a relatively broad, elliptical portion proximal to the NE. In some micrographs it appeared attached to the NE by fine struts. The smaller, layered, cylindrical distal portion appeared connected to the proximal portion by an amorphous substance. Several MBs and a small zone of exclusion surrounded each SPB. Microtubules (MTs) appeared to originate near the zones of exclusion and increased in number between SPBs as one SPB migrated to establish the other division pole.

By metaphase the proximal portion of each SPB disappeared, while the distal SPB portion remained at the fenestrated poles and became closely associated with the intranuclear spindle MTs (Fig. 1c). Most MTs bypassed the chromatin, but several MTs were seen between the poles and chromatin, inserted on indistinct, but identifiable kinetochores (Fig. 1d).

During early anaphase the chromatin moved to the poles followed by a pronounced elongation of the interzonal spindle. The distal SPB portion and some extranuclear MTs were still present in early interphase.

It is understandable that kinetochores and SPBs were concluded to be absent in *Porphyridium*, although both appear to be evident in the published micrographs¹. It

was initially difficult to locate them even though we examined serial sections of many dividing cells. Kinetochores, because of their size and inconspicuous structure, were rarely encountered; and while SPBs were frequently observed in prophase, they were easily overlooked in subsequent mitotic stages when only the distal portion remained.

It is not possible to assign functional attributes to the MBs associated with the nuclear poles. This feature, considered to be unique to *Porphyridium*¹, has been observed in unicellular green algae where it is believed that the MBs are simply

taking advantage of the spindle to ensure equal allocation to each daughter cell (K. Stewart, personal communication). We support this idea concerning the polar distribution of MBs because they are not known to be implicated with spindle formation or function and because there are other spindle-associated organelles which are now thought to be non-functional in nuclear division and are instead taking advantage of a 'free ride'^{2,3}.

Based on our observations, we conclude that the word 'unusual' is inappropriate and misleading to describe mitosis in *Porphyridium*, and should be used in

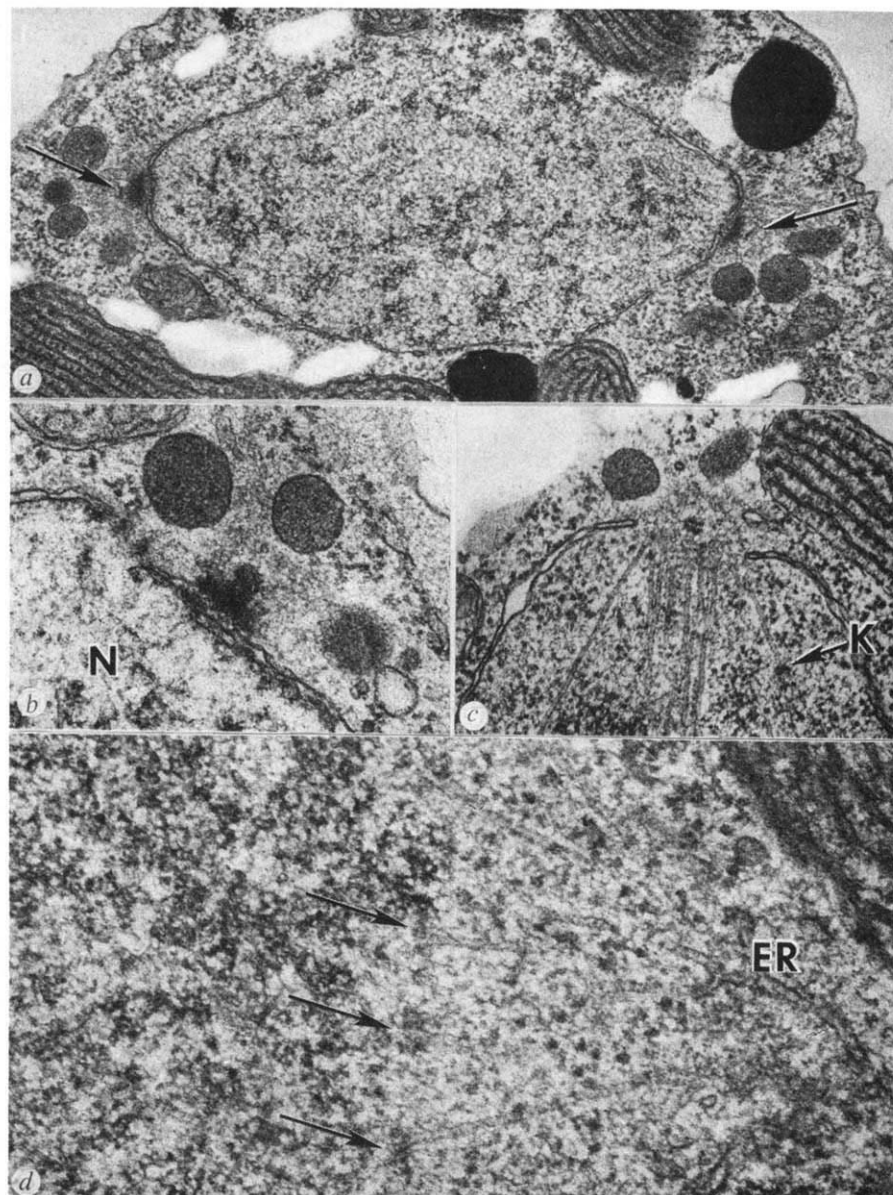


Fig. 1 a, Longitudinal view of late prophase nucleus showing established division poles. Each SPB (arrows) bridges a slight NE depression. The intranuclear spindle has not formed yet. The NE of this cell was apparently disrupted during specimen preparation, for it is well preserved in most cells ($\times 33,750$). b, Early prophase SPB. Note its association with the NE and MBs. Few MTs are present near the small zone of exclusion at this time. N, nucleus ($\times 67,500$). c, Distal portion of SPB at metaphase. K, kinetochore ($\times 42,000$). d, Tangential polar view of a metaphase nucleus showing kinetochores (arrows) with attached MTs. Endoplasmic reticulum (ER) is often seen at the poles ($\times 90,000$).

characterising organisms whose mitotic mechanisms resemble those currently found only in primitive forms such as the hypermastigote flagellates, dinoflagellates and certain other protozoa⁴. Also, while there are minor modifications, the fundamental characteristics of mitosis in Bangiophyceae and Florideophyceae red algae appear to be the same (ref. 5 and our work in preparation with C. Bosco and J. Thomas). Furthermore, it has been suggested that an interrelationship exists between red algae and certain fungi^{6,7}. The presence of morphologically similar, NE-associated SPBs in both groups, along with other common ultrastructural cell division features^{4,8} may serve to strengthen this idea.

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BRONCHART AND DEMOULIN

REPLY—Schornstein and Scott have added some interesting observations to our data on mitosis in *Porphyridium*, and their interpretation of spindle pole body behaviour is especially welcome. Their main criticism of our paper seems to concern the use of the word 'unusual', a very subjective point. Our use of that word is due to the association of microbodies with the spindle poles, a fact confirmed by Schornstein and Scott. Neither unpublished reports of such a situation in some other algae nor speculation about its meaning change our opinion that our use of that adjective was legitimate. Our use of the word unusual did not result from a belief that either kinetochores or spindle pole bodies were absent.

Kubai¹ gives a lengthy table (Table 6) of organisms displaying microtubules terminating in chromatin without differentiation of kinetochores. We are aware that improved fixation and staining, or better luck in obtaining the right section, may eventually have revealed in those organisms, structures that might be described as kinetochores. Thus we did not stress the fact that we had not seen them in *Porphyridium*.

There is nothing unusual in the absence of a spindle pole body which is the rule among higher plants, but, as we said in our report, we observed and photographed (Fig. 2) such an organelle in *Porphyridium*. We spoke, however, of a microtubule-organising centre, for we were sure only of its presence in prophase and were not certain of its existence at the spindle poles in metaphase. We did not feel the small

dark masses present at the poles in metaphase (our Fig. 1 and Fig. 1c of Schornstein and Scott) could qualify as a 'well defined pole body'. It is to the merit of Schornstein and Scott to have shown their constancy and relation to part of the prophase organelles. Those observations however do not make mitosis in *Porphyridium* closer to that in *Membranoptera*², because except for a qualification of the kinetochore situation (kinetochores are well defined in *Membranoptera* and inconspicuous in *Porphyridium*) every difference we noted remains true (presence of microbodies; lack of polar ring, well condensed chromatin and nuclear envelope doubling by endoplasmic reticulum). As to the relationship to fungi, there is certainly some similarity to the situation in Basidiomycetes. We already had that feeling in 1977 but did not stress it for fear of being accused of stretching the evidence in favour of a red algal ancestry for higher fungi, to which one of us is devoted.

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Cancers and the immune system

EXCEPTIONALLY high levels of antibodies against the Epstein-Barr virus have been found in the blood of children who later developed African lymphomas¹, and this finding is widely regarded as evidence of a viral origin for these unusual tumours². However, the tumours are little more than exceptionally localised (and easily cured) lymphatic neoplasms, and they are all but confined to areas of holo-endemic malaria where the diffuse disease (lymphatic leukaemia) is conspicuous by its absence. In these same regions, over 50% of childhood cases of myeloid leukaemia have localised collections of myeloid cells (chloromas³) as the presenting symptom. Therefore, we could be witnessing the effects of general constraints on cancers of lymphatic and haematopoietic tissues in regions where a combination of high infection risks and low standards of nutrition makes the acquisition of exceptionally high levels of immunological competence during infancy a condition of further survival.

This hypothesis assumes that although the immune system may have more difficulty in recognising quasi-foreign cells (for example, mutants) than wholly foreign cells (microorganisms), nevertheless, it is playing essentially the same role in infective and neoplastic diseases. Therefore, an appropriate test would be one which compared the illnesses and

immune reactions of European or American children with lymphatic leukaemia and more localised lymphatic neoplasms such as lymphosarcoma and Hodgkin's disease.

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DE THÉ AND GESER REPLY—The comments of Stewart and Kneale on the contrast between the preponderance of localised lymphomas in tropical climates versus diffuse lymphoid malignancies in temperate climates are interesting. The prospective study carried out from 1972 to 1978 in Uganda showed that up to 4 years before Burkitt's tumour development, the future cases were marked by high antibody titres against Epstein-Barr virus (EBV)-specified viral capsid antigens, when compared with the general child population of the same age, sex and locality. The study further showed that other herpes virus antibody levels were not elevated in the Burkitt's lymphoma (BL) candidates and we consider that this specific stimulation of EBV antibody production contradicts the view implied by Stewart and Kneale that BL is caused by an exceptionally high level of immunological competence during infancy, rather than by a specific EBV-related event. As discussed elsewhere¹, Burkitt's lymphoma develops, as do most cancers, as the result of a multi-stage process, each step having specific causes. That high levels of immunological stress could promote the development of localised lymphatic neoplasms (BL) rather than leukaemias as seen in temperate climates, is an old and conceivable hypothesis, which does not contradict the involvement of a specific initiating viral event, as proposed for BL.

We agree with Stewart and Kneale that a comparative epidemiological, clinical and immunological study of lymphatic leukaemias versus localised lymphosarcomas, in temperate and tropical regions, would answer the question of whether children with localised lymphomas are more immunologically competent than those with diffuse lymphoid malignancies.

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REVIEWS

Impact of microelectronics

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THE subtitle of this book, *Forecasting the Effects of Information Technology*, though less eye-catching than the main title, gives a better impression of its content. Here, in fairly raw form, is a report to Her Majesty's Government's Department of Industry. It contains the "intelligent extrapolations" of the two authors concerning probable impact of microelectronics over the next fifteen years. The Department certainly got what it needed. The authors have grasped the nettle firmly and given some serious criticisms combined with some quite radical recommendations for change in policy. To put their criticisms in a nutshell, the authors say that, so far, there has been talk only about the impact of microelectronics and that any practical moves made by the Government have all been small and poorly directed. As a result Britain, which has the capability of being in the top three is, in fact, seventh amongst the countries of the world in terms of progress in the use of microelectronics. The report provides a substantial number of the tools necessary to guide the Government in correcting this state of affairs.

In the 'Information Revolution' mankind has been granted a boon that it does not yet quite recognise. Swamped with information, man can now handle it at a low cost and with such ease that his life may greatly increase in quality. Unfortunately many see silicon microprocessors and memories as a threat rather than a boon. The revolution will impinge on business, on the factory floor, and on the home. Large sections of certain industries (for example, those making meters and electromechanical devices) may become redundant and this, if handled wrongly, could obviously be traumatic. Later on, even paper could be largely replaced by silicon. The benefits will lie in increased prosperity; a reduction of the anxiety felt today as we are presented with more information than we can handle; a greater opportunity for people to use their intelligence rather than their brawn; and better use of increasingly scarce material resources. The book under review does not spend time delineating these benefits. Its object is to describe how to reap them. Not only does it provide the forecasts demanded but, even more usefully, it details how Britain can improve its position in the field, using well reasoned argument.

The Future with Microelectronics. By Iann Barron and Ray Curnow. Pp. 243. (Frances Pinter: London, 1979.) £7.95.

One example of a tight technical argument is that provided on a strategy of production of silicon devices. The authors suggest that, within a short time, a generally adequate level of performance in integrated circuits will have been reached. When that point has been reached, British manufacturers should not continue trying to surpass their competitors in device performance but should instead attempt to extend their markets by a reduction in the price of the same devices. The authors note that this can best be done by reducing the number of rejects and devising a less labour-intensive way of encapsulating the silicon chips. By these means, a British firm could prosper against foreign competition. Such ideas might seem to represent nothing but conventional manufacturing commonsense but, for an industry so wedded to development of the silicon part of their product, the ideas have a revolutionary ring. Such arguments make it clear that at least one of the authors has considerable experience in the business of making microelectronic devices. Civil servants will be advised to listen well to such people before deciding policy. Makers of policy will also be helped by the effort, comprehensively made here, to carry their technical arguments logically into the sphere of industrial aid policy. Perhaps one of the most important items in the report is a recommendation that the government shift their financial support away from research and towards assisting the actual sales of devices; moreover, the authors recommend that support be shifted away from Government research establishments and towards industry laboratories. I would imagine that the aim of this recommendation is not primarily to put an axe into research but rather to increase substantially the whole level of Government funding of microelectronics, keeping the research level constant but helping to promote specific ranges of the equipment which has been improved by the use of microelectronics. Such a direction could, the authors hold, much more surely increase Britain's share of the market in several highly profitable fields, such as office equipment (the authors see clerical work as one of the fields most

prone to be changed by microelectronics in the near term). Again, the idea might seem obvious did not the authors note that many Government committees find it more congenial to fund research than to support the manufacture of end-products. Though stated kindly and factually, the implication of the authors is clear: some of our civil servants have, in this field, tended to steer clear of hard decisions which involve the fate of a business. I would welcome a longer book which includes some discussion as to why this should be so, and how British practice compares with that in, say, France and the USA. Is it a lack of technical appreciation of the microelectronics industry, aggravated, perhaps, by the great speed with which it is developing? Or is it a fundamental characteristic of civil servants to avoid the more critical gambles? If so, will a report, like this, however convincing, conquer that tendency?

For the general reader, the main problem with this book will come with the style, which is that of a technical report. In the interests of bringing the report quickly to a wider public, it would seem that little time was spared for making it more intelligible to that public. The one superficial addition, a glossary of technical terms, is a particularly weak effort. In fact, many definitions miss the point and some of the more obscure terms in the text are not covered. Moreover, the authors sometimes favour Americanisms (what is an "implementation house"?) and adjectival phrases ("handling, machining, forming and labour intensive"). It is a pity that an important statement of the future, as I believe this is, may only further baffle the general public.

Does the book give a well-rounded picture of the next fifteen years or more? Here, it may be said that the authors' immersion in one technology may work against them. They are clearly "silicon men" and claim that silicon devices of one class, n-channel MOS devices fabricated from single-crystal silicon, can answer all of our needs in microelectronics, at least in the medium term. They therefore give virtually no time to considering the potential technical advances in other branches of semiconductor technology, such as that of gallium arsenide and certain thin-film structures. Even in the medium term, such devices could possibly both outperform *and* underprice silicon

devices. I would most certainly agree that there is ample room for the expansion of the use of silicon devices in low-speed microelectronics (for example, a silicon diary could inform one of tomorrow's appointment and still operate at a very low data rate); but other growing fields, such as mass data-processing, demand such high switching speeds that the designers of fast computers already claim that silicon is "running out of capabilities". This, however, is one of the few points which is insufficiently argued in a book which, without doubt, is an important contribution to the debate on Britain's

role in the Information Revolution. The highly condensed statement which they give leaves room for several further books which expand on the issues so succinctly raised and it is to be hoped that this statement will be a first step in a wider, more sophisticated, debate on the impact of microelectronics on our future lives. □

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Ageing research

I. Davies

The Biology of Senescence. Third edition. By A. Comfort. Pp.414. (Elsevier: New York, 1979.) \$19.95.

THE study of ageing is now taking place on a large scale throughout the world. There is a rapid proliferation of new books with "overviews" of the subject to date. The second edition of Dr Comfort's book was produced in 1964 and at that time was one of the few texts analysing senescence in a variety of animal species, and also presenting a critical survey of current theory in the field of ageing research. One rather expected that the latest edition of this book would attempt to carry out the same task, but with an updated review of

the current literature. However, the end product is rather a strange hybrid, a very thorough analysis of early research in the ageing field, followed by a somewhat scanty examination of the later literature.

Much of the text is easily recognised as being from the earlier edition and contains an interesting description of the historical and philosophical background of the study of the ageing process. There is a mass of data on the maximum life-spans achieved by animals from most of the major phyla, together with discussions of the various measurements (morphological, biochemical, physiological, and so on) made on such ageing populations. The influence of genetic constitution and growth on senescence is also described. Much of this information is valuable and informative, but the majority of the references cited are pre-1964 with hardly any reference to the wealth of recent data.

Various other defects are also apparent — such as non-matching of references in

the text with those in the extensive bibliography, and also examples of changes in symbols between diagram and legend — but presumably these can be blamed on the publishers.

The remainder of the book examines the proposed mechanisms of senescence, a discussion of the position of ageing studies, and a call for the direct measurement of human ageing. This is the most recent section of the text and contains a very brief review of current concepts in the field of gerontology. The discussion is too short and is not critical enough. In addition, there is some rather blatant grafting into the previous edition with a close juxtaposition of statements such as "antibodies are now thought to be produced by lymphocytes" and then more or less detailed descriptions of current research on immunological factors in the ageing process. The scantiness, and the choice, of the references used to question (for example) the blind acceptance of senile neurone loss, is also doubtful. Similar criticism could be levelled at other topics.

Overall, I found this book very disappointing. There is a good deal of useful information which would be of use in establishing background for graduate course work in a study of senescence. However, because of the less than satisfactory updating of the book it would be of limited use for a review of current research into the ageing process. □

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Basic physics of X-ray spectroscopy

D.S. Urch

X-Ray Spectroscopy: An Introduction. By B.K. Agarwal. Pp.418. (Springer: Berlin, Heidelberg and New York, 1979.) DM74; \$40.70.

X-RAY SPECTROSCOPY is enjoying a renaissance with the development of techniques such as extended fine structure (EXAFS), with the availability of high-resolution spectrometers and with the construction of powerful sources of synchrotron radiation. It is therefore a pleasure to welcome Professor Agarwal's new introduction to the subject. Of necessity much of the book is concerned with the basic physics of X-rays and this involves the presentation of a considerable amount of historical material. This is done in a clear and logical way with full mathematical derivations. Indeed, if there is a general criticism of this book, it is that there is

possibly an over-emphasis on mathematical rigour at the expense of descriptive explanation.

The first three chapters are devoted to a description of X-electromagnetic radiation, to characteristic X-rays and to the interaction of X-rays with matter. This latter subject then leads on to specific topics of current interest. The value of the book is greatly enhanced at this stage by a consideration of the photoelectric and Auger effects, and the consequences which these processes have for X-ray spectroscopy. Peak widths and the origin of the non-diagram (satellite) lines are dealt with in detail. As high resolution X-ray spectroscopy in both emission and absorption can shed considerable light on the electronic structure of metals, alloys and other solid materials as well as molecules, this 'chemical' application is described in some depth. The various types of chemical bond are described and there is a brief introduction to molecular orbital theory so that chemical shifts of X-ray emission peaks can be rationalised. The fine structure near X-ray absorption edges is discussed in the same way and a basic account is given of the

EXAFS technique and of the information that can be derived from it. Even so, it is unfortunate that in this chapter (as in others) very few recent references are to be found (less than 10% of the references are post-1970). This lack is particularly felt in the chapter on the fast growing area of soft X-ray spectroscopy where fundamental advances are either omitted (for example, Henke X-ray tube) or barely mentioned (for example, grazing incidence spectrometer).

General experimental methods in X-ray spectroscopy are dealt with quite briefly, only the basic physical principles being explained in detail. The emphasis in this book is theoretical, rather than experimental, but it has merit as an introduction to the basic physics of X-ray spectroscopy and to its applications. It should therefore form a valuable addition to the libraries of X-ray laboratories and could be used in postgraduate courses in X-ray physics. □

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Soluble cell products in the immune response

Anne S. Hamblin

Biology of the Lymphokines. Edited by S. Cohen, E. Pick and J.J. Oppenheim. p.626. (Academic: New York, San Francisco and London, 1979.) \$42.50; £27.60.

THE word lymphokine was originally invented to denote those cell-free soluble factors, other than immunoglobulins, generated *in vitro* during the interaction of sensitised lymphocytes with specific antigen, and with biological activities consistent with them functioning as regulators of immune responses *in vivo*. Subsequent work has shown that the same, or similar, activities may be produced from other cells of both lymphoid and non-lymphoid origin under a variety of circumstances. The editors have therefore taken the view (with which there might be some disagreement) that their terms of reference for topics discussed in this book should be wide, and include "soluble cell products of all categories to which a function in the central or peripheral regulation of the immune response has been attributed, with the exception of classical antibodies". Within the potentially enormous list of substances which could be discussed in this context,

the editors have made a clearly stated selection of those which will receive attention and those which will not. This book is therefore about the activities of some soluble cell products, mostly derived and demonstrable *in vitro*, which are at present considered relevant to the regulation of immune responses *in vivo*. However, it is not simply a list of factors, but more interestingly an attempt to focus on some aspects of current lymphokine research, and in particular mechanisms of action.

The book consists of twenty chapters by different authors, most of whom have considerable experience of experimental work with lymphokines. The first, by the editors, entitled "The lymphokine concept", clearly explains the problems surrounding the definition, origin and relevance of lymphokines, and sets the stage for the ensuing chapters. The second entitled "Lymphokines as inflammatory mediators", includes a useful discussion of all the available, compelling, although inconclusive, evidence that these substances, the activities of which are based on *in vitro* assays, do have a role *in vivo*, and provides a basis for argument with those who regard lymphokines as test-tube artifacts. Thereafter follow sixteen chapters, some of which review 'classical' lymphokine activities (migration inhibitory, cytotoxic and mitogenic factors) and some of which describe more recent fashionable additions to the lymphokine repertoire (factors affecting T cell - B cell cooperation, antigen-specific regulatory factors, and specific and non-specific suppressor T cell

factors). In addition, there is an interesting chapter by L. Epstein comparing the properties of classical and immune interferons, a chapter on the important and difficult problems of purification and characterisation of lymphokines, and several chapters on lymphokine activities derived from a variety of cell sources of both lymphoid and non-lymphoid origin. The book concludes with two general reviews; the first a stimulating article on the role of intracellular mediators in the immune response by C. Parker, which although it makes little reference to lymphokines, was presumably included on the basis that the editors feel that future research on their biology may focus on such mechanisms; and the second an overview of the biology of lymphokines by B. Waksman.

The topics selected for inclusion in this book reflect current aspects of the study of lymphokines which the editors view as important. Within our present state of limited knowledge about soluble cell products involved in immune regulation, and in particular their biochemical purification and characterisation, the relative importance of the subjects discussed remains a matter of speculation. Nonetheless, the book brings together information and concepts which can form the basis of useful discussion amongst those actively involved in lymphokine research, as well as those more generally interested in biological control mechanisms. □

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Simple qualitative molecular orbital theory

Anthony Stone

Molecular Structure and Bonding: The Qualitative Molecular Orbital Approach. By B. M. Gimarc. Pp. 224. (Academic: New York, San Francisco and London, 1979.) £11.70.

THIS book endeavours to explain how a knowledge of simple qualitative molecular orbital theory can give a detailed understanding of the shapes of molecules. It is rather limited in scope, covering only H_3 , H_4 , AH_2 to AH_4 , AB_2 to AB_6 , HAB and H_2AB , A_2H_2 , A_2H_4 and A_2H_6 , and dimers of AB , AB_2 and AB_3 (where A and B are first or second row elements; there is no mention of transition-metal compounds) but these molecules are discussed in considerable detail, with many references to experimental and *ab initio* data. The book is aimed at senior undergraduates or first-year graduates, though it is not

entirely consistent in level: for example, it is not thought necessary to explain what is meant by the Hartree-Fock method with configuration interaction, but the simple molecular-orbital picture of H_2 is discussed in some detail. Similarly the reader is not expected to know or learn any group theory beyond "a knowledge of symmetry classifications", but the molecular orbitals of the various species are given, in qualitative form, without any hint of the group-theoretical procedures used in their derivation. Such theoretical explanation as is given (for example, of the change in the form of the orbitals on bending on AH_2 molecule) is woolly and unconvincing. Students who had not met Walsh diagrams before would have difficulty with the treatment of AH_2 , let alone the more complicated examples.

However, the main limitation of this book is its single-minded commitment to one approach. Changes of energy on passing from one structure to another are to be viewed wholly in terms of changes in overlap. There is no mention, in the main text, of Walsh, whose classic series of papers dealt with most of these molecules 25 years ago, or of the Jahn-Teller effect or the pseudo-Jahn-Teller effect. The only

concession to other methods of tackling these problems is a couple of pages at the end of the book, in which other approaches are briefly mentioned, almost as if to counter the accusation that the author has never heard of them, but there is no attempt whatever to integrate these ideas into the book as a whole. In the discussion of H_4 for example, three possible structures are considered, namely tetrahedral, square-planar and linear. The overlap criterion is used to rank these in order of energy, and it is concluded, not very convincingly, that the linear structure lies lowest. But application of the Jahn-Teller theorem shows conclusively that the ground state *cannot* be tetrahedral or square-planar, because either of these structures would, according to the theorem, distort spontaneously. The reader should be told about this far more rigorous approach.

In summary, the book contains a good deal of useful information on a rather restricted subject, but it is discussed from a very narrow point of view. A disappointing book. □

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Blood-brain barrier

Stanley I. Rapoport

The Concept of a Blood-Brain Barrier. By Michael Bradbury. Pp.465. (Wiley: Chichester, UK, and New York, 1979.) £22.

THIS book by Michael Bradbury, of King's College, London, is a scholarly, comprehensive and readable analysis of past and recent studies on the blood-brain barrier (BBB) by a researcher who has made major contributions to the field. It extends the important review by Davson (*Physiology of the Cerebrospinal Fluid*, Churchill, Livingstone: Edinburgh, 1967) and complements, with only slight overlap, my own book (*Blood-Brain Barrier in Physiology and Medicine*, Raven: New York, 1976). It contains more than 1,000 references, and should be of value to researchers as well as to neuropharmacologists, neurochemists, neurosurgeons, neurologists and transport physiologists.

The BBB separates the brain and cerebrospinal fluid (CSF), on the one hand, from blood on the other, and is a regulatory system consisting of three tissue sites — the cerebrovasculature, the choroid plexus and the arachnoid membrane. Each site contains at least one cell layer that is connected by tight junctions (which restrict intercellular diffusion of water-soluble drugs and proteins) and that has specific transport mechanisms that help to regulate the ionic and water contents of the brain, and to facilitate delivery to the brain of critical substrates for metabolism and synthetic processes.

Chapter 1 introduces the anatomical and functional relationships between barrier tissue sites and brain cells. Chapter 2 summarises early studies on the barrier to intravascular dyes and colloids, and presents methods of compartmental analysis that can be used to estimate cerebrovascular permeability. The vasculature is selectively permeable to lipid-soluble as compared to water-soluble agents. Chapter 3 reviews historical controversies about BBB sites, and shows that the BBB at the cerebral vasculature arises at the continuous layer of endothelial cells that have little pinocytosis and are connected by tight junctions. The chapter also analyses methods of measuring cerebrovascular permeability *in vivo*, and discusses capillary hydraulic and water permeability. Bradbury concludes that functional pores exist at the vascular endothelium for non-electrolyte diffusion, although I believe that evidence suggests that they do not. The sink action of CSF, in washing material from the brain extracellular space, is discussed.

Chapter 5 indicates that apparent regional differences in blood-brain exchange reflect differences in capillary surface area between white and grey

matter. It considers the special, high permeability regions in the brain in detail (that is, median eminence, posterior pituitary), which have vessels with porous, fenestrated endothelia and have neuroendocrine and chemoreceptor functions. Blood vessels in the retina, optic nerve and peripheral nerve also have a continuous endothelial lining.

Chapter 6 elaborates the properties of saturable, stereospecific mechanisms at cerebral capillaries for facilitated diffusion of sugars, amino acids, ketone bodies and monocarboxylic acids. Bradbury suggests that amino acids are transported actively from brain to CSF, although the evidence for this is weak. Chapter 7 critically evaluates experiments designed to prove active transport at the cerebral capillary, and finds most of them wanting. The best one is that by Davson and Hollingsworth with ^{131}I .

Chapters 8 and 9 summarise much of Bradbury's original studies on homeostatic regulation of ions within the nervous system. The apparent selectivity of the cerebral capillary to K^+ as compared to Na^+ could be due to active K^+ transport or to exchange diffusion of K^+ . Transport at the choroid plexus maintains CSF concentrations of Ca^{2+} , Mg^{2+} , Na^+ and K^+ within strict limits, despite marked changes in plasma concentrations. A sigmoid relationship between CSF K^+ and K^+ efflux at the plexus, and a K^+ -independent influx, could stabilise CSF K^+ . Brain and CSF pH are also stabilised through modification of choroidal secretions and brain organic ion content (especially in alkalosis). Control of brain ionic content helps to maintain a

constant brain volume during a chronic change in plasma osmolality.

BBB phylogeny is discussed in Chapter 10. BBB structures are in place but are less tight in the immature than in the mature brain. Regulatory mechanisms for specific ions mature at different rates, but are completely developed during the final spurt of brain growth.

Chapter 11 discusses the BBB to drugs, neurotransmitters and metabolites. Most current methods are not sufficiently sensitive to measure capillary permeability of slowly permeating agents, such as peptides. Clinical depression and schizophrenia are related to CSF concentrations of specific neurotransmitters or their metabolites. CSF concentrations of folic acid, vitamin C, peptides and morphine are regulated by choroidal transport.

Chapter 12 summarises effects of different insults on BBB permeability — hypercapnia, seizure, hypertension, toxic agents, hypertonic solutions. Bradbury concludes that the possible mechanisms that might increase cerebrovascular permeability — tight junctional widening, increased vesicular transport, trans-endothelial channel formation — remain to be evaluated critically. The resistance of the BBB to hypoxia and ischaemia is emphasised, as are principles underlying cerebral oedema. Chapter 13 summarises overall BBB function. A final proof reading of the book would have avoided a few trivial errors. □

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Birdwatching postscript

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The Mitchell-Beazley Birdwatchers' Pocket Guide. By Peter Hayman. Pp. 192. (Mitchell-Beazley/RSPB: London, 1979.) £3.95.

THIS is a slender, wallet-sized book which can be conveniently carried with one into the field. It is crammed full of coloured sketches, most of which are very small (less than an inch across), but they usefully portray the birds in a variety of plumages and poses (perched and in flight) and from different angles. Juvenile plumages are also often shown, though I would have preferred more of these to have been included.

The text is very sparing but includes the most important features of each species, their calls, their habitats, and range. Some of this information is given in the form of

symbols, thereby saving space. There is also usually a small silhouette of each species against that of either a sparrow or a pigeon in order to give a quick idea of size. This is not a total success; when the bird in question is very large compared with scale model (for example, pheasant with sparrow or eagle with pigeon) the model may be less than 2 mm in size. In such cases, the poor reader is left to determine which of these two models this tiny spot of ink is supposed to represent!

The book covers some 350 species and hence includes almost all the birds of Western Europe. More detail is given for those which are commonest in Britain than for the rarer birds; the latter are sometimes grouped together on a page and so are not located with their closest relatives. The order used is not an accepted taxonomic one, but one designed to help the beginner locate the species concerned. On the whole this is a well produced, highly portable little book which many will find very useful. □

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